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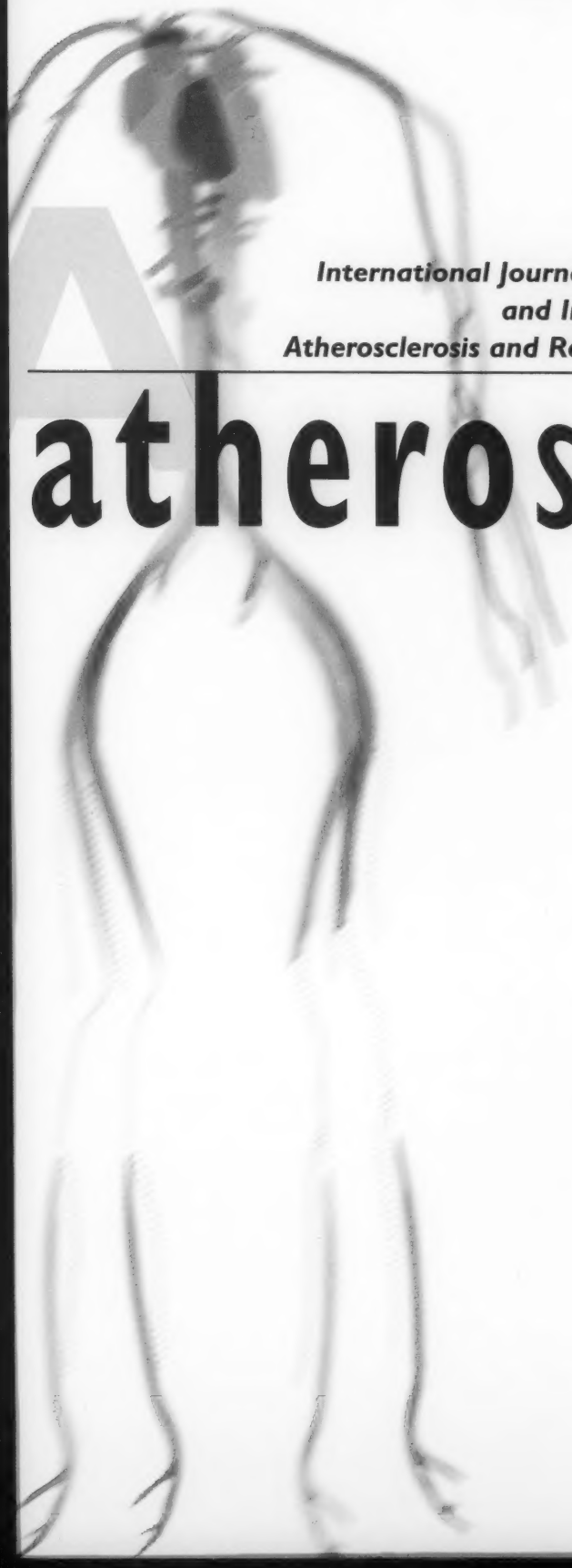

International Journal for Research and Investigation on Atherosclerosis and Related Diseases

atherosclerosis

Affiliated with the International Atherosclerosis Society

Supplement
68th Meeting of the European Atherosclerosis Society
Molecular Cell Biology and Atherogenesis
Brugge, Belgium
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Atherosclerosis brings together from all sources papers concerned with research and investigation on atherosclerosis, its complications and related diseases, including: lipoprotein metabolism, arterial and vascular biology and disease, thrombosis, inflammation, disorders of lipid transport, diabetes and hypertension as related to atherosclerosis, and cardiovascular risk factors. The editors are also interested in clinical papers dealing with case studies of specific or general interest, new or unusual lipid syndromes, and the genetic basis and familial incidence of atherosclerosis and related diseases. High quality reports of controlled clinical trials of drugs or diets will be considered provided the paper deals with the mechanism of action of the drug or diet.

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Abstracts

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1

Atherosclerosis and the two faces of endothelial nitric oxide synthase (NOS III).

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Nitric oxide (NO) has been shown *in vitro* to interfere with key events in the development of atherosclerosis. *In vivo* NO activity is a balance between the synthesis of NO and breakdown of NO. Support for a defect in NO production mainly derives from observations that administration of L-arginine, the substrate for NOS III, improved NO-dependent vasodilation in atherosclerosis and that L-arginine could prevent development of experimental atherosclerosis. This led investigators to conclude that a reduction in NO synthesis, due to a reduced availability of substrate is the primary process involved in endothelial dysfunction. However, the intracellular concentration of L-arginine by far exceeds the K_m of NOS, making the possibility that L-arginine is rate-limiting less likely. In agreement, using 15N-L-arginine kinetics in hypercholesterolemic subjects we only find a small reduction in *in vivo* NO synthesis in these patients.

Alternatively, reduced NO activity could be caused by enhanced catabolism. The *in vivo* half-time of NO is mainly determined by its reaction with oxy-hemoglobin and superoxide. The reaction of superoxide and NO occurs at a diffusion-limited rate, with the production of the powerful oxidant peroxynitrite. The endothelium is an important source of superoxide which appears to be increased in hypercholesterolemia. Importantly, NOS III itself can also be an important source of endothelial superoxide production in hypercholesterolemia. Reduced co-factors induce a sequential transfer of electrons in the enzyme to the heme group. During this electron transfer both FAD/FMN as well as the heme group may reduce oxygen and form superoxide. The electrons may also be donated directly to the aminoguanidine group of L-arginine, which will result in the formation of NO and L-citrilline. Another co-factor, tetrahydrobiopterin (BH4), has been postulated to play an important role in directing this electron flow towards L-arginine. This effect of BH4 may be related to its effect on the allosteric conformation of the enzyme. Preliminary data from our department confirm that BH4 stabilizes recombinant NOS III in its dimeric form, and that BH4 reduces superoxide production and increases NO production by the enzyme. In agreement, we recently demonstrated in hypercholesterolemic patients that intra-arterial administration of BH4 restored the impaired NO-dependent vasodilation. It is possible that the abnormal intracellular redox state in atherosclerosis, which is unfavorable for reduction of the oxidized biopterin, impaires the endothelial recycling of BH4. This concept emphasizes modulation of the altered redox state in atherosclerosis by e.g. antioxidants as a therapeutic strategy early in the development of atherosclerosis.

2

The oxidation hypothesis of atherosclerosis.

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Oxidized LDL and auto-immune antibodies against oxidized LDL have been demonstrated in atherosclerotic lesions. In vitro experiments have demonstrated that they may enhance the progression of those lesions by 1) inducing endothelial dysfunction; 2) enhancing monocyte adhesion and foam cell generation; 3) enhancing platelet adhesion and aggregation; 4) impairing vasodilatation; and 5) impairing the anticoagulatory and fibrinolytic activity of the arterial wall.

The internal area of fibrofatty plaques in the coronary arteries of cholesterol-fed New Zealand White rabbits and of Watanabe Heritable Hyperlipidemic rabbits correlated with levels of oxidized apo B-100 containing lipoproteins, VLDL and LDL. Balloon angioplasty in the carotid arteries of these hypercholesterolemic rabbits induced proportional neointimal thickening and accumulation of oxidized VLDL/LDL. The progression of both hypercholesterolemia-induced lesions in the coronary arteries and of angioplasty-induced lesions in the carotid arteries were due to smooth muscle cell proliferation and not to monocyte/macrophage accumulation.

The hypothesis that oxidized LDL play a role in the pathogenesis of coronary artery disease in heart transplant patients was investigated. Plasma levels of oxidized LDL were measured with a monoclonal antibody (mAb-4E6) based enzyme linked immunosorbent assay that had a 1000-fold higher sensitivity for oxidized LDL than for native LDL. Mean levels of oxidized LDL were significantly lower in patients with angiographically normal coronary arteries than in patients with mild or severe coronary artery stenosis. Logistic regression analysis revealed that plasma levels of oxidized LDL and age of donor predicted the development of coronary artery disease in these patients. Plasma levels of oxidized LDL and of von Willebrand factor were also significantly increased in chronic renal failure patients. Multiple regression analysis revealed that the extent of renal failure accounted for a significant fraction of the variation in oxidized LDL levels even after exclusion of patients with evidence of ischemic atherosclerotic disease. The levels of oxidized LDL corelated with the levels of von Willebrand factor. Thus, these data demonstrated that oxidized LDL increase progressively during the development of renal failure and suggested that the oxidation of LDL may be associated with endothelial injury.

In conclusion, although the present data do not allow to conclude that oxidized VLDL and LDL play a causal role because the oxidation of those lipoproteins may be a consequence of a more generalized oxidative abnormality, they are certainly in agreement with a model in which radical oxidative stress contributes to the progression of cardiovascular disease.

3

Macrophages and LDL oxidation

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Oxidized LDL was shown to play a major role in macrophage foam cell formation, the hallmark of early atherosclerosis. Macrophages can oxidize LDL and this process involves activation of cellular oxygenases, as well as macrophage antioxidants.

We have previously demonstrated the role of macrophage NADPH oxidase in cell-mediated oxidation of LDL. The possible role of cellular glutathione in protecting LDL from oxidation by macrophages was also studied. Reduction of cellular glutathione in J-774 A. 1 macrophage-like cell line, or in mouse peritoneal macrophages (MPM), by cells incubation with botionine sulfoximine (BSO), an inhibitor of glutathione synthesis, significantly increased (by about 3 fold) macrophage-mediated oxidation of LDL. In contrast, a 60% elevation in macrophage glutathione content (by cells incubation with a cystein donor, OTC), resulted in up to 40% inhibition in cell-mediated oxidation of LDL. Similarly, treatment of the atherosclerotic, apolipoprotein E-deficient (E°) mice with selenium (1 µg/mouse/wk for a period of 6 months) resulted in a 44% inhibition in the ability of their peritoneal macrophages to oxidized LDL, paralleled by a 30% reduction in the size of their atherosclerotic lesion.

In addition to macrophage antioxidants, LDL-associated antioxidants such as combination of vitamin E, β-carotene and lycopene, but mainly the polyphenols quercetin (from red wine) or glabridin (from licorice), were shown to inhibit the susceptibility of LDL to oxidation, both in humans and in the E° mice.

In conclusion then, intervention means to increase macrophage glutathione content or LDL-associated polyphenols, can significantly attenuate LDL oxidation, and hence, may arrest the development of accelerated atherosclerosis.

4

Effect of Glycated LDL on endothelial free radical release and its relevance for vascular dysfunction

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Glycation of proteins and lipoproteins caused by hyperglycemia is known to grossly impair endothelial/smooth muscle vascular function and to increase the risk for atherosclerosis. This study was therefore designed to investigate how glycated LDL (gLDL) affects endothelial cell vascular function.

Preheatment of porcine aortic endothelial cells with gLDL (100 µg cholesterol/ml; app. 12% glycation) enhanced superoxide anion (O_2^-) release to 383% (measured by the SOD-inhabitable reduction of ferrocytochrome c). Treatment with gLDL, isolated from a diabetic patient (HbAlc = 9.4%) enhanced endothelial inositol 1,4,5-trisphosphate (IP_3) synthesis, Ca^{2+} signaling (fura-2) and, thus, EDRF/NO formation to bradykinin (conversion from L-^{3}H-arginine to L-^{3}H-citrulline) by 87, 76 and 42 %, respectively. The potency of gLDL to evoke O_2^- release and changes in signal transduction pathways (IP_3, Ca^{2+} and NO) correlated with the amount of LDL glycation. While the aldose-reductase inhibitor, sorbinil had no effect, SOD, vitamin E, GSH and probucol diminished the stimulatory effect of gLDL on O_2^- release and signal transduction pathways (IP_3, Ca^{2+} and NO). Convincingly, the effect gLDL on the parameter measured were mimicked by xanthine oxidase/ hypoxanthine, a O_2^- generating system. These data indicate that gLDL-associated changes in Ca^{2+} signaling, IP_3 formation and EDRF/NO, as such considered as beneficial, are linked, on the other hand to the appearance of O_2^-.

Beside these effects of gLDL on endothelial vascular function, the enhanced O_2^- release yielded in a 42% loss of NO bioactivity to stimulate cGMP formation in a target cell by 42%. Hence, preincubation of endothelial cells and smooth muscle cells almost doubled NO-scavenging by cellular-derived radicals.

On the other hand, gLDL inhibited L-arginine uptake depending on the amount of its glycation (up to 68%), resulting in a decrease in intracellular L-arginine level by 37%. Since endothelial NO formation critically depends on intracellular L-arginine, it is tempting to assume that over a long period of time, gLDL might attenuate NO formation, due to lacking enzyme substrate.

Thus, gLDL affects endothelial cellular signal transduction mechanisms, free radical release, NO degradation and NO scavenging, L-arginine uptake and, perhaps in a long term, NO formation. We believe that these effects contribute to the pathophysiology and vascular complications in diabetes mellitus.

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5

Lipoprotein receptor gene expression in atherosclerotic lesions

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Lipid-loaded cells are the hallmark of atherosclerotic lesions. Lipid-filled foam cells are derived from monocyte/macrophages and smooth muscle cells. At least four major lipoprotein receptors have been characterized that could be involved in arterial lipid uptake. These receptors include: scavenger receptor(s), LDL receptor-related protein/α2-macroglobulin receptor, LDL receptor and VLDL receptor. With the exception of the LDL receptor, all of these receptors are expressed in atherosclerotic lesions. Whereas scavenger receptors are mostly expressed in macrophages, the LDL receptor-related protein may play an important role in mediating lipid uptake in smooth muscle cells. Additional receptors that could mediate lipoprotein binding and/or uptake are CD36, CD68, Fc receptor and putative receptor(s) for oxidized LDL. It appears that no single receptor pathway is solely responsible for the increased lipid uptake in lesion cells, but several redundant mechanisms may contribute to the uptake and degradation of lipoproteins in atherosclerotic lesions.

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Ylä-Herttuala S. Expression of lipoprotein receptors and related molecules in atherosclerotic lesions. Current Opin. Lipidol. 7:292-297, 1996.

6

Drugs and LDL oxidation

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The peroxidation step of lipid transformation is considered to be essential in the pathogenesis of atherosclerosis. The clinical implications of free radicals and antoxidants in this disease as well in other ones are widely discussed in the scientific literature and lay press. Although data concerning the mechanisms by which lipid peroxidation occurs in vivo are scarce, several lines of evidence suggest that some endogenous and exogenous compounds with antioxidant activity could have some beneficial effects in the prevention of atherosclerosis. Ascorbic acid (vitamin C) and α-tocopherol (vitamin E) act as the most important hydrophilic and lipophilic antoxidants respectively in vivo. Accordingly, animal and human studies suggest that these compounds may have some preventive effect against the development of clinical coronary heart disease. Many plant phenols and flavonoids may be important dietary antioxidants and it has been speculated that these compounds in red wine or in the Mediterranean diet could explain the << French paradox >>. Several studies show that antioxidants such as probucol and butylated hydroxytoluene can inhibit development of atherosclerotic lesions in Watanabe and cholesterol-fed rabbits.
Some drugs such as β-blockers, calcium antagonists, hypolipodemic drugs, ... appear to have at least in vitro antioxidant effects but the clinical relevance of these properties remains unkonwn. Moreover, some interventions aimed to decrease the LDL-oxidative susceptibility have not been shown to attenuate atherogenesis when cholesterol levels remain markedly elevated.

7

Cytokines in atherogenesis

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The cytokines are multipotent mediators of inflammation and immunity that can affect key functions of vascular wall cells. Growing evidence suggests that cytokines may participate as autocrine or paracrine mediators in atherogenesis as cells in lesions can produce as well as respond to these mediators. The functions of vascular wall cells regulated by cytokines may influence lesion initiation, progression, complication. For example cytokines can regulate the expression of adhesion molecules crucial to the recruitment of leukocytes to lesions, including vascular cell adhesion molecule-1 (VCAM-1). Cytokines such as interleukin 1 (IL1) and tumor necrosis factor (TNF-α) can regulate the production of monocyte chemoattractant protein-1 (MCP-1), a potential signal for directed migration of monocytes into the intima. Cytokines can also regulate genes that encode other growth factors and cytokines themselves. TNFα can induce IL1 mRNA in human endothelial (EC) and smooth muscle cells (SMC). IL-1 and TNF-α can augment the production by vascular cells of macrophage-colony stimulating factor (M-CSF), which may promote growth and activation of mononuclear phagocytes. Cytokines can exert both pro- and anti-atherogenic actions. Although some cytokines stimulate cell proliferation, the T-cell-derived cytokine IFN-γ can inhibit SMC proliferation which may reduce fibrogenesis. However, in conjunction with IL-1 and TNF-α, IFN-γ can induce SMC apoptosis, an effect that might impair plaque stability. In addition to soluble cytokines such as IL-1 and TNF, more recently characterized cell surface molecules such as the TNF family members CD-40 and Fas may also alter functions of vascular cells and leukocytes related to atherosclerosis. Thus, expression of cytokines can influence multiple aspects of atherogenesis, and provide new and interesting targets for therapeutic intervention.

8

Epidemiological aspects of HDL

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The distribution of high density lipoprotein cholesterol (HDL-c) has been described in different populations. Results from ecologic studies suggest that HDL-c is important as a protective factor in populations with elevated coronary heart disease (CHD) rates but less so in societies with low CHD incidence. In Western populations HDL-c is strongly determined by sex but also by various lifestyle factors such as physical activity, smoking and nutrition. Qualitative aspects of fat consumption, alcohol and certain micronutrients have been associated with the HDL-c distribution. Results from the Belgian Interuniversitary Research on Nutrition and Health show a strong negative association between the P/S ratio of the diet and HDL-c in both sexes, independent of other determinants such as smoking, alcohol, BMI, diabetes, systolic blood pressure and the use of anticonceptives.

HDL-c levels can be influenced by certain antihypertensive drugs, hormones and lipid lowering drugs.

In most case-control and prospective cohort studies a strong inverse relationship has been observed between HDL-c and CHD although weakened after adjustment for associated factors.

Post hoc analysis of randomized controlled trials have shown indirect evidence of a preventive role by changing HDL-c in primary and secondary prevention of CHD.

9

High density lipoproteins and reverse cholesterol transport. Lessons from natural mutations

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The reverse cholesterol transport model is widely used to explain the protective role of HDL in atherogenesis. HDL encompass structurally and functionally heterogeneous particles. Two-dimensional nondenaturing polyacrylamide gradient gel electrophoresis and subsequent immunoblotting helps to differentiate quantitatively minor HDL-subclasses from the bulk of HDL, which contains apoA-I and has electrophoretic α-mobility. Pulse-chase experiments identified the quantitatively minor HDL subclasses preβ$_1$-LpA-I, γ-LpE, and LpA-IV as initial and fast acceptors of cell-derived cholesterol and α-migrating HDL (i.e. α-LpA-I) as a late and slow acceptor. In plasmas of patients with certain forms of familial HDL-deficiency such as apoA-I deficiency and Tangier disease, preβ$_1$-LpA-I, γ-LpE, and LpA-IV represent the only HDL particles and account for the significant residual cholesterol efflux capacity of these plasmas. These particles however also fulfil important roles in reverse cholesterol transport of normal plasma. Preβ$_1$-LpA-I, for example, is generated, during the interconversion of HDL by lipid transfer proteins. Thus incubation of plasma with phospholipid transfer protein increases the concentration of preβ$_1$-LpA-I and in parallel increases the cholesterol efflux capacity of plasma indicating that lipid transfer proteins modulate cholesterol efflux by modification of HDL subclass composition.

ApoE and γ-LpE are of special interest for reverse cholesterol transport since macrophages can produce apoE. We investigated the impact of apoE polymorphism on the ability of human macrophages to express apoE mRNA and secrete apoE. A reverse transcription-polymerase chain reaction assay with a competitive internal RNA standard did not identify genotype-related differences in apoE mRNA expression in unloaded cells. Under this condition apoE3/3 cells secreted approximately two-fold more apoE than cells which did not express apoE3. Loading with acetyl-LDL led to genotype-related increases in apoE mRNA in the order apoE4 > apoE3 > apoE2 and in apoE secretion in the order apoE4 > apoE2 > apoE3. Moreover, only the medium of apoE3 producing macrophages contained a particle with the mobility of γ-LpE. Whether these genotype-related differences in synthesis, secretion, and formation of apoE-containing particles by human macrophages cause differences in cholesterol efflux from macrophages is currently under investigation.

10

Molecular mechanisms of reverse cholesterol transport

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The participation of HDL in the reverse cholesterol transport of cholesterol from peripheral cells to the liver is critical for the antiatherogenic properties of this lipoprotein. Experimental results showing that efflux of cholesterol from cells growing in culture is mediated by HDL, and lipoprotein particles containing apo A-I in particular, support this conclusion. A bidirectional flux of unesterified cholesterol molecules between the plasma membrane of cells and HDL particles in the extracellular medium occurs. Net efflux of cholesterol mass from the cells involves passive diffusion of cholesterol molecules through the aqueous phase and down their concentration gradient between the plasma membrane and HDL; the concentration gradient is maintained by LCAT-mediated esterification of cholesterol molecules in the HDL particles. Fully lipidated apo A-I is important in promoting this "aqueous diffusion" mechanism because it (1) acts as a cofactor for LCAT and (2) solubilizes phospholipid into small HDL-sized particles that are efficient at absorbing cholesterol molecules diffusing away from the cell surface. Apo A-I also exists in an incompletely lipidated state in plasma. Apo A-I molecules in this state are able to solubilize phospholipid and cholesterol from the plasma membrane of cells. This "membrane-microsolubilization" process is enhanced by enrichment of the plasma membrane with cholesterol and is the mechanism by which pre-β-HDL particles in the extracellular medium remove cholesterol and phospholipid from cells. The relative contributions in vivo of the "aqueous diffusion" and "membrane-microsolubilization" mechanisms of apo A-I-mediated cell cholesterol efflux are not predicted readily from cell culture experiments. Confounding issues are the variation with cell type and the dependence on the degree of cholesterol loading of various domains in the cell plasma membrane.

11

Abstract not Received

12

Plasma LCAT and CEPT activity during lipid lowering therapy

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Lecithin:cholesterol acyltransferase (LCAT) esterifies free cholesterol acquired by (nascent) HDL particles and is essential for their conversion to mature HDL's. LCAT plays a crucial role in one of the initial steps of reverse cholesterol transport from peripheral cells to the liver for excretion. Cholesteryl ester transfer protein (CETP) is facilitating exchange/transfer of cholesteryl esters, triglycerides and phospholipids between lipoproteins. In particular, cholesteryl esters in HDL may be exchanged for triglycerides in VLDL. The importance of LCAT or CETP activity as risk factors for atherosclerosis in man is difficult to assess. A positive correlation between LCAT and plasma LDL is present in healthy subjects and partial LCAT deficiency in Fish Eye Disease may cause severe, early coronary artery disease. Low CETP levels have been linked with longevity as well as with atherosclerosis. A major effect of CETP may be on the cholesteryl ester content of VLDL and LDL, as is suggested by its high activity level (measured with exogenous substrates, an estimate of CETP mass) in dyslipidemia due to apoE defects. These mechanisms make it important to monitor changes in LCAT and CETP during lipid lowering therapy.

Dietary factors like fatty acid profiles are important in determining plasma CETP (but not LCAT) activity levels in normolipidemic healthy subjects. CETP levels are lowered in hypercholesterolemic patients treated with simvastatin. Interestingly, literature data suggest that pravastatin treatment does not affect CETP levels. CETP as well as LCAT activity levels are not decreased in hypertriglyceridemic patients treated with gemfibrozil: in fact both plasma CETP mass and activity levels were found to be increased. It must be noted that in vivo endogenous substrate lipoproteins are also important for LCAT and CETP action. This is clear from the observation that, probably due to the lowering of plasma triglycerides in VLDL, the cholesteryl ester transfer rates, measured with endogenous lipoprotein substrates, are decreased by fibrates. Long-term, prospective studies are necessary to find out whether plasma CETP or LCAT mass and activities are indicative for the development of atherosclerosis in man.

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Regulation of APO A-I gene expression by fibrates

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Fibrate hypolipidemic drugs are important modulators of plasma high density lipoprotein (HDL) concentrations, which are inversely correlated to the development of atherosclerosis. In rodents, fibrates decrease HDL cholesterol by lowering the transcription of its major apolipoprotein apo A-I in liver. In contrast, in man, fibrates increase plasma levels of HDL and apo A-I. Fibrates induce human apo A-I gene expression by activation of the transcription factor PPARα (peroxisome proliferator-activated receptor) which interacts with a specific response element, termed PPAR-responsive element (PPRE), in the human apo A-I gene promoter. The lack of induction of apo A-I expression by fibrates in rodents is due to single nucleotide differences in the rodent apo A-I promoter eliminating binding of PPAR and activation by fibrates. These *in vitro* observations were extended in vivo in transgenic mice and rabbits overexpressing the human apo A-I gene under control of its homologous promoter containing the PPRE. Whereas the endogenous mouse apo A-I gene is repressed, treatment with fibrates results in the transcriptional induction of human apo A-I gene expression in the transgenic mice. This induction is accompanied by an increase of human apo A-I containing HDL in plasma. These *in vivo* data demonstrate that the opposite effects of fibrates on apo A-I gene expression in rodents and humans is due to differences in regulatory elements in their respective genes. In order to determine whether fibrates could increase HDL and apo A-I concentrations without inducing an hepatomegaly and peroxisome proliferation, their effects were tested in rabbits, an animal model resistant to peroxisome proliferation. In contrast to normal rabbits, where plasma lipoprotein levels remained unchanged upon fibrates, treatment of transgenic rabbits resulted in increased plasma HDL and human apo A-I concentrations. This induction is associated with increased human apo A-I mRNA levels in liver, whereas rabbit apo A-I gene expression remains constant. In conclusion: 1) Fibrates increase plasma HDL concentrations in humans, at least partly, due to the induction of apo A-I gene transcription. 2) Solely the presence of the human apo A-I gene is sufficient to confer fibrate-responsiveness on HDL. 3) The beneficial effects of fibrates on lipoprotein metabolism are independent of any undesirable proliferation of peroxisomes .

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Drug control of reverse cholesterol transport

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Reverse cholesterol transport is the process by which excess cholesterol in peripheral tissues, including the arterial wall, is transported to the liver for excretion. High density lipoproteins (HDL) and their major protein component, apolipoprotein A-I (apoA-I), play a central role in this process, a function believed to explain the strong inverse correlation between plasma HDL levels and the incidence of coronary heart disease observed in many case-control and prospective studies. Therefore, therapeutic interventions aimed at stimulating this transport process may hold promise in the prevention and treatment of arterial disease. Among the drugs affecting lipoprotein metabolism, only fibrates and probucol can exert some effects on reverse cholesterol transport, by increasing the expression of HDL apolipoproteins and of cholesteryl ester transfer protein, respectively. Another approach to the stimulation of reverse cholesterol transport, made possible by the tremendous progresses in the recombinant DNA technology, consists of transiently raising plasma HDL levels, either by exogenous supplementation of synthetic HDL or by endogenous overexpression of HDL apolipoproteins through gene-therapy. The infection of animals with recombinant viruses effectively raised plasma apoA-I and HDL levels, but the present vectors need to be improved to achieve sustained expression and alter atherosclerosis progression. Synthetic HDL containing extractive apoA-I, recombinant proapoA-I or disulphide-linked apoA-I_{Milano} dimer proved effective in preventing the development of vascular lesions in various animal models of human atherosclerosis. The first studies in humans demonstrate that such treatment is feasible, safe and effective in raising plasma HDL levels. While awaiting clinical studies evaluating the effects of synthetic HDL on vascular lesions, further progresses in this area would require a better understanding of the structure-function relationship in apoA-I, with the aim of identifying specific domains responsible for the apoA-I function in reverse cholesterol transport, as well as for the ancillary properties of this protein, like prostacyclin stabilisation, stimulation of fibrinolysis and inhibition of cell-cell interactions. This would lead to the development of novel rnolecular entities with improved anti-atherosclerotic activity.

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***Trans* fatty acids and HDL**

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The effects on serum total cholesterol concentrations of dietary *trans* fatty acids from hydrogenated oils have been investigated for many years. Results of the earlier studies, however, are conflicting. In addition, effects of *trans* fatty acids on the distribution of cholesterol over the various lipoproteins were not studied.
Recent studies have shown that *trans*-C18:1 as found in hydrogenated vegetable oils increases concentrations of the atherogenic LDL-cholesterol. *Trans*-C18:1 do not have a marked effect on HDL-cholesterol as compared with carbohydrates, while most other fatty acids in the diet increase HDLcholesterol.
One postulated mechanism for these effects is that *trans* fatty acids increase the activity of the plasma cholesterylester transfer protein (CETP). CETP exchanges cholesterylesters from HDL for triglycerides in LDL and VLDL. Indeed, it has been demonstrated that *in vitro* the activity of CETP was significantly higher after replacement of oleic acid for trans-C18:1. In addition, a decrease in the ratio of cholesterylesters to triglycerides in HDL has been reported. This finding indicates that the increased CETP-activity as observed in in vitro also affects lipid transfer reactions *in vivo*.
Although results from prospective epidemiological studies suggest that a high intake of trans fatty acids increase CHD-risk, it is not known whether and to what extent the effects of *trans* fatty acids on HDL-cholesterol contribute to this effect

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Contribution of the C-terminal helix of apo A-II to the displacement of apo A-I from HDL.

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Computer modelling of the apo A-II 53–70 and 58–70 C-terminal segments, suggests that these amphipathic helices are oriented at an angle of 30° at a lipid/water interface, due to the C-N hydrophobicity gradient along the helix. Mutant peptides were designed by computer modelling to be either parallel (0°) or perpendicular (90°) to a lipid bilayer, and the capacity of the WT- and variant peptides to induce fusion of pyrene-labeled phospholipid vesicles was measured. The excimer/monomer fluorescence ratio decreased down to 35 and 65% of its initial value for dimyristoylphosphatidylcholine (DMPC) vesicles, compared to 65 and 85% for pyrene-labeled phosphatidylcholine/phosphatidylethanolamine/cholesterol (PC/PE/chol) vesicles with the WT apo A-II 53–70 and 58–70 peptide respectively. None of the mutant peptides had any fusogenic activity. Vesicle fusion was further accompanied by an increase of the size of the pyreneabeled DMPC vesicles, after mixing with the WT apo A-II peptides, whereas the mutants had no effect. The membrane destabilization properties of the apo A-II peptides were monitored by measuring the release of calcein, entrapped inside PC/PE/chol vesicles, upon addition of the apo A-II peptides. Calcein leakage amounted to 55% for apo A-II 53–70 and 20% for apo AII 58–70, whereas it was negligible for the mutant peptides.
We further investigated the role of the C-terminal helical segments of apo A-II (residues 53–70 and 58–70) and their mutants in the displacement of apo A-I from HDL_3, by incubating HDL_3 with increasing amounts of the apo AII peptides. Gel filtration on a Superose 12 PG column, demonstrated that the amount of apo A-I released from HDL_3 increased with the peptide/apo A-I ratio. A maximal displacement of 55% apo AI was observed for the WT apo A-II 53–70 peptide, compared to 20% for the apo A-II 58–70 peptide. The corresponding 0° and 90° mutants were unable to displace apo A-I from HDL3.
These data suggest that the angle of insertion of the C-terminal apo A-II helical peptide into a lipid bilayer, is critical for both its fusogenic activity and its ability to displace apo A-I from HDL_3. In native HDL, the tilted insertion of the C-terminal helix of apo A-II resulting in a partial destabilization of the HDL external lipid layer, might account for the displacement of apo A-I by apo A-II

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The pharmacological control of the mevalonate pathway regulates arterial smooth muscle cell proliferation

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The role of mevalonic acid (MVA) and its products (isoprenoids) in cell proliferation prompted us to investigate the effect of drugs affecting diverse enzymatic steps of the MVA pathway on rat aorta smooth muscle cell (SMC) proliferation. To evaluate this parameter, synchronized SMC (2×10^5 cells/dish) were incubated in a medium containing 10% FCS with the tested drugs for 72 hours. SMC proliferation was measured by cell count after trypsinization of the monolayers, using a Coulter Counter. Competitive inhibitors of HMGCoA reductase (statins) decreased SMC proliferation in a dose-dependent manner. The inhibitory effect induced by simvastatin 3.5 μM (30% ± 3.8 of control cells) was prevented by the addition of 100 μM MVA (100% ± 2.3), 10 μM farnesol (F-OH) (85% ±1.2) and 5 μM of all-*trans* geranylgeraniol (GG-OH) (precursors of prenylated proteins) (81% ± 1.1), but not by 2-*cis* GG-OH (precursor of dolichols), squalene and ubiquinone. Squalestatin 1 (1–25 μM), a potent squalene synthase inhibitor, blocked cholesterol synthesis and slightly decreased (79% of control cells) SMC proliferation only at the highest tested concentration. NB-598 (1–10 μM), a potent squalene epoxidase inhibitor, caused a full inhibition of cholesterol synthesis, measured by the incorporation of [^{14}C]acetate into cellular sterols, without affecting SMC proliferation. Finally, BZA-5B (10-50 μM), a specific inhibitor of protein farnesyltransferase (PFTase), inhibited SMC proliferation in a dose- and time-dependent manner (38% ± 6.3 of control cells) after 9 days of incubation with the highest concentration, without affecting cholesterol synthesis. This effect was partially prevented by MVA (76% ± 3.2) and all-*trans* GG-OH (87% ± 7.3) but not by F-OH. BZA-5B was also able to inhibit by 50% [^{3}H]F-OH, but not [^{3}H]GG-OH incorporation into specific cell proteins. On the other hand, SMC proliferation was not affected by the closely related compound BZA-7B, which does not inhibit PFTase. Taken together, these findings support the involvement of specific isoprenoid metabolites in cell proliferation and suggest the MVA pathway as a possible target for the pharmacological control of cell growth.

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Lifibrol (K12.148): First member of a new class of hypolipidemic drugs?

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Lifibrol (4-(4'-tert-butylphenyl)-1-(4'-carboxyphenoxy)-2-butanol) is a new hypocholesterolemic compound. It effectively lowers total cholesterol, low density lipoprotein (LDL) cholesterol, and apolipoprotein (apo) B. In addition, Lifibrol reduces lipoprotein (a), serum triglycerides, and fibrinogen.

In the hepatoma cell line HepG2, Lifibrol decreased the formation of sterols from [^{14}C]-acetic acid by 20 and 30 % when used at concentrations of 10^{-6} mol/l and 10^{-5} mol/l, respectively. Similar to Lovastatin, Lifibrol had no effect on the synthesis of sterols from [^{14}C]-mevalonic acid. Lifibrol did not inhibit 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase. Instead, cholesterol synthesis inhibition by Lifibrol was entirely accounted for by competitive inhibition of HMG-CoA synthase. Lifibrol enhanced the cellular uptake and degradation of LDL in cultured human skin fibroblasts in a dose dependent fashion. The stimulation of LDL receptors was significantly stronger than expected from the effect of Lifibrol on sterol synthesis. In parallel to the receptor mediated endocytosis of LDL, Lifibrol increased the amounts of LDL receptor mRNA and of immunologically detectable receptor protein. We are currently investigating the effects of Lifibrol on the transcriptional activation of sterol-dependent genes using reporter gene constructs harboring the 5'-regulatory region of the LDL receptor gene and the HMG-CoA synthase gene, respectively. Preliminary results indicate that Lifibrol enhances transcriptional activity in this system. In patients with heterozygous familial hypercholesterolemia Lifibrol (600 mg per day) increased the fractional catabolic rate of LDL 2.3 fold. Lifibrol reduced the production of LDL apo B by approximately 50% and enhanced the production of high density lipoprotein apo AI by 50%. A patient homozygous for a cysteine by tyrosine substitution at residue 307 of the LDL receptor (less than 5% receptor activity *in vitro*) did not respond to Lifibrol, indicating that the action of Lifibrol crucially depends on functional LDL receptors. In contrast to fibrates, Lifibrol did not affect the expression of acyl-CoA-oxidase and apo CIII via the peroxisome proliferator-activated receptor (PPAR) in HepG2 cells. In rats, Lifibrol had no effect on the concentrations of the cholesterol precursors desmosterol and lathosterol, but markedly reduced the concentrations of the phytosterols sitosterol and campesterol. In humans, Lifibrol decreased both cholesterol precursors and plant sterols, the effect on plant sterols being stronger than on cholesterol precursors.

The present data suggests that Lifibrol acts synergistically upon key regulatory processes of cholesterol homeostasis: It reduces cholesterol absorption from the intestine, moderately decreases hepatic cholesterol biosynthesis and stimulates the expression of low density lipoprotein receptors, presumably by a sterol independent mechanism; it does not act on PPAR dependent genes. Lifibrol thus sets up a new class of lipid modulating compound, clearly distinct from HMG-CoA reductase inhibitors and fibrates, and appears to be a versatile agent in treatment of hyperlipidemia.

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Cholesterol-Lowering Independent Effects of Pravastatin on the Artery Wall of Atherosclerotic Primates.

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Recent evidence indicates that pravastatin may reduce the risk of coronary heart disease independent of its effects on plasma cholesterol concentrations. To examine these lipid-independent effects, 32 adult male cynomolgus monkeys were fed an athero genic diet for 4 years (progression phase). Monkeys were then fed a lipid lowering diet for an additional 2 years that contained (n = 14) or did not contain (n = 18) pravastatin (20 mg/kg body weight/day) in the diet (regression phase). As intended in this study design, and as expected in this animal model, total plasma cholesterol and high density lipoprotein cholesterol concentrations and blood pressure were similar between groups during both the progression and regression phase of the experiment ($p > 0.05$). Histomorphometric analyses of arteries indicate no treatment effects on mean plaque size in coronary arteries at the end of the regression phase (0.110 ± 0.048 vs 0.125 ± 0.05 mm^2), or in reduction of plaque size ($48 \pm 5\%$ vs $45 \pm 6\%$) of the iliac artery during the regression phase of the experiment (control vs pravastatin, $p > 0.05$). Quantitative angiography indicated that coronary arteries of pravastatin-treated monkeys dilated ($10 \pm 3\%$) whereas those from control animals constricted ($-2\% \pm 2\%$) in response to infusion of acetylcholine ($p < 0.05$). There were no treatment effects on coronary responses to nitroglycerin. Histologic grading of arteries indicated that there was a shift in frequency distribution towards fewer macrophages, less calcification, and less neovascularization in the intima of the pravastatin-treated monkeys compared to the control monkeys ($p < 0.05$ for all measures). Thus, while pravastatin had no appreciable effects on reduction in lesion size, endothelium-mediated dilator responses of coronary arteries were greater in pravastatin-treated monkeys. Furthermore, treatment with pravastatin resulted in a favorable shift away from lesion characteristics (presence of macrophages, calcification and neovascularization) that have been associated with plaque instability. We conclude that pravastatin has favorable effects on both arterial function and plaque stability characteristics independent of its effects on plasma lipoprotein concentrations and blood pressure.

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Pravastatin protects against development of chronic rejection in rat cardiac allografts and upregulates immunosuppressive TGF-B2

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Purpose: Based on the observation that transplant cardiac vasculopathy is inhibited by pravastatin in human clinical trials; the immune modulatory effect of this HMG-CoA reductase inhibitor drug was tested in a rat cardiac transplant model of chronic rejection.

Methods: Cardiac rat Lewis-F344 heterotopic abdominal transplant model was used with transient cyclosporine (CsA) immunosuppression (10 days) with high (10 mg/kg) or low (5 mg/kg) dose oral pravastatin (Prav) for 120 days. The effect of pravastatin was analyzed by histology, immunohistochemistry, and northern blot analysis of transforming growth factor B2 (TGF-B2), a cytokine with immunosuppressive properties. Cryostat sectioned tissue was H&E stained and graded for coronary vasculopathy by a pathologist (0 = normal and 5 = complete luminal occlusion). Immunohistochemistry for extracellular matrix (ECM) proteins, laminin (LN) and fibronectin (FN), which may play a regulatory role in lymphocyte migration, was performed using polyclonal antibodies and biotinylated secondary antibody with alkaline phosphatase reaction. Northern blot analysis was performed with TGF-B2 simian cDNA probe.

Results: Coronary artery vasculopathy was inhibited in both sets of pravastatin treated animals. Either dose of pravastatin also decreased myocardial necrosis and infiltration of inflammatory cells. Immunohistochemistry revealed that the CsA only treatment animals

	CsA only	Low Prav	High Prav
arterial vasculopathy[a]	2.8 ± .8	1.3 ± .4	0.7 ± .4
myositis	+++	++	+
myocardium necrosis	+++	+	–
intermyocyte FN/LN	–	+	+
degradation FN/LN	+++	+	–
	(n=6)	(n=4)	(n=2)

[a]mean ± SD; $p < 0.05$ for each group comparison.

had marked degradation of LN and FN, whereas the pravastatin treated groups had modest amounts of ECM proteins retained within intermyocytes and endothelium; a pattern seen in normal tissue. Northern blot analysis with TGF-B2 demonstrated overexpression of this cytokine in pravastatin treated animals.

Conclusion: Progressive chronic vascular rejection, a leading cause of allograft failure, can be inhibited in a dose dependent fashion by pravastatin in a rat cardiac transplant model. Pravastatin appears to inhibit synthesis and subsequent degradation of.ECM proteins. In addition, upregulation of TGF-B2 with pravastatin suggests an immunoprotective role of this cytokine on transplant chronic rejection, in contrast to producing fibrosis, as has been postulated previously.

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Lipid changes and reductions in the incidence of major coronary heart disease events in the Scandinavian Simvastatin Survival Study (4S)

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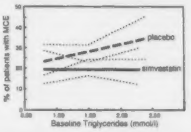
4S demonstrated a significant 30% reduction in all-cause mortality ($p = 0.003$) due to a 42% decrease in coronary mortality ($p < 0.00001$) in 4,444 post-MI and angina patients treated with simvastatin (S) for a median of 5.4 years. Major coronary events (MCE), defined as CHD death and non-fatal MIs, were reduced by 34% ($p < 0.00001$). Excluding the 2% of patients with extreme values, prior to randomisation, total cholesterol (TC) ranged from 5.0 to 8.0 mmol/L, low-density lipoprotein-cholesterol (LDL-C) from 3.0 to 6.5 mmol/L and high-density lipoprotein-cholesterol (HDL-C) from 0.7 to 2.1 mmol/L, whereas triglycerides (TG) were < 2.5 mmol/L. Higher baseline TC ($p = 0.013$) and LDL-C ($p = 0.024$), and lower baseline HDL-C ($p = 0.036$), were associated with increased risk within the P group. As evident in other studies, baseline TG levels were also significantly associated with the risk of MCE in the P group ($p = 0.007$). In the S group the relationship with baseline lipids were less strong and significant for HDL-C ($p = 0.026$) and Apo B ($p = 0.039$) only.



Mean percent changes in TC, LDL-C, TG and HDL-C were −25%, −35%, −10% and +8%, respectively, over 5.4 years. Changes in the placebo (P) group were minimal, except for a 7% increase in TG. Percent changes in TC ($p < 0.0001$), LDL-C ($p < 0.00001$) and HDL-C ($p = 0.038$) were related to the reduction in MCE in the S group (with adjustment for other risk factors). However, there was no independent relationship between percent TG reduction in the S group and reduction in MCE risk ($p = 0.37$). Most of the effect of S was explained by the reduction in LDL-C. No threshold level for LDL-C was detected below which benefit was no longer obtained.

Collectively, these findings underscore the importance of lowering LDL-C in order to reduce CHD risk. Baseline TG was associated with increased risk in the P group, but not in the S group. However, there was no evidence in 4S that lowering TG was beneficial, suggesting that TG is a marker for risk but not a causal factor.

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Cholesterol Lowering Efficacy of Simvastatin at Doses of 80 and 160 mg/d in a new low systemic exposure formulation

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To reach target levels, patients with CHD and hypercholesterolemia often require large LDL-C reductions. Because greater drug plasma levels increase the potential for adverse effects, especially at high doses, a new formulation (GEM--Gel Extrusion Module) has been developed that slows delivery of simvastatin into the gut and substantially reduces drug plasma levels.

To evaluate the efficacy and safety of simvastatin GEM, 141 subjects with LDL-C > 160 mg/dl and triglycerides (TG) < 350 mg/dl were randomized to simvastatin at doses of 80 and 160 mg once daily, with the 80 mg conventional tablet (CT) as a control, in a 18 week, double-blind, 2 period, incomplete block cross-over study. Each active treatment period was 6 weeks in duration with an intervening 2 week wash-out period. Median % changes from baseline were:

	Baseline (mg/dl)	80 mg/d CT	80 mg/d GEM	160 mg/d GEM
N	141	89	85	87
Total-C	305	−35	−35	−40
LDL-C	221	−47	−48	−55
TG	183	−21	−24	−30
apo B	202	−40	−41	−46
HDL-C	49	9	9	12
apo Al	151	11	12	10

No patient developed myopathy; 2 patients taking 80 mg CT had hepatic transaminase elevations > 3X the upper limit of normal. No new or unexpected adverse effects were observed. We conclude that the GEM formulation provides equal efficacy with plasma levels lower than with the conventional formulation and a low short-term incidence of adverse effects; our results support the continued investigation of simvastatin GEM.

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Atorvastatin reduces lesion size and macrophage content in a rabbit model of atherosclerosis, and atenuates NFκB activation and MCP-1 expression in cultured VSMC.

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Acute ischemic events are most frequently due to plaque disruption and thrombosis. HMG-CoA reductase inhibitors reduce total and coronary mortality, both in primary and secondary prevention, though the mechanism is not completely understood. It is known that lipid rich and highly macrophage (Mø infiltrated plaques are more prone to rupture. In a rabbit model of atherosclerosis we have studied the effect of atorvastatin (Atv), a new HMG-CoA-reductase inhibitor, on the lesion size, its Mø content and the expression of monocyte chemoattractant protein 1 (MCP-1). Atherosclerosis was induced in the femoral arteries by endothelial damage (nitrogen desiccation) and an atherogenic diet (0.5% cholesterol, 14% coconut oil) given during 4 weeks. Then, animals were switched to standard rabbit chow, randomized to receive either no treatment (n = 8) or Atv (5 mg/Kg/day) (n = 7) and sacrificed after 4 weeks. Lesion size was determined by morphometry and arterial Mø infiltration by immunohistochemistry (RAM-11). Atv reduced total cholesterol (60%), LDL (40%) and triglycerides (50%) ($p < 0.04$). Lesion size was reduced considering both the intima/media ratio (0.129 ± 0.038 *vs* 0.412 ± 0.322; $p = 0.001$) and the maximal stenosis ($10 \pm 7\%$ *vs* $21 \pm 13\%$; $p = 0.038$). Mø infiltration was present in the untreated group (4/7 animals; mean Mø neomtimal area $1,470 \pm 916$ μm^2), and this was completely abolished by Atv treatment (0/8 ammals; $p = 0.034$). MCP-1 mRNA expression (RT-PCR) was 2-fold higher in untreated *vs* Atv-treated rabbits. The presence of MCP-1 protein in the neointima (immunohistochemistry) was significantly reduced by Atv treatment ($3,881 \pm 1,473$ *vs* $41,657 \pm 14,380$ μm^2; $p < 0.0002$). This reduction (90%) cannot be explained solely by the diminution in lesion size (50–60%). In order to study a potential direct effect of Atv on the arterial wall, we determined Atv effect on MCP-1 expression and nuclear factor κB (NFκB) activation (a transcription factor that controls MCP-1 expression) in cultured vascular smooth muscle cells (VSMC). Atv reduced MCP-1 expression (20–35%) and NFKB activation (45–70%) ($p < 0.05$) induced by angiotensin II, tumor necrosis factor-α and interleukin-1β, mediators involved in the pathogenesis of atherosclerosis. In conclusion, in a model of atherosclerosis, Atv reduces lesion size, Mø infiltration and MCP-1 expression. The *in vitro* experiments suggest an additional direct effect of Atv on VSMC independently of plasma lipid reduction. Since Møs are implicated in plaque rupture, the diminution in neointimal Mø infiltration by Atv may contribute, at least in part, to the beneficial effect of statins found in clinical trials.

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HMG-CoA reductase inhibition by atorvastatin (ATV) induces apoptosis of vascular smooth muscle cells (VSMC) in culture — role of isoprenoids of the mevalonate pathway

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Intimal thickening, an early feature of atherosclerosis, is the result, in part, of migration, proliferation and eventual death (including programmed cell death) of VSMC from the media. HMG-CoA reductase inhibitors (statins) inhibit VSMC migration and proliferation in vitro. However, the potential effects of statins on VSMC apoptosis have not been evaluated in spite of the fact that statins induce apoptosis in a variety of cell lines. We thus studied whether HMG-CoA reductase inhibition by ATV modulates apoptosis of VSMC in culture. Exponentially growing rat VSMC (4th–12th passage) were exposed to increasing concentrations of ATV (10–100 μM) for 24h in the presence of 10% fetal bovine serum as a survival factor. The presence of apoptosis was evaluated by morphological criteria: 1) cell rounding, blebbing and detachment from substrate (contrast phase microscopy) 2) chromatin condensation and fragmentation (fluorescence microscopy after DNA staining with bisbenzimide), 3) quantified as the proportion of cells exhibiting a hypodiploid DNA content (flow cytometry after VSMC permeabilization and DNA staining with propidium iodide), and confirmed by 4) increased binding of FITC-annexin V (by cytofluorography). ATV induced VSMC apoptosis in a dose-dependent manner (% of apoptosis: control 2.6 ± 2.2; 100 μM ATV 17.6 ± 2.4 $p < 0.05$). Lipophilic statins also induced VSMC apoptosis in a dose-dependent manner (100 μM lovastatin $46.1 \pm 0.3\%$, 100 μM simvastatin 33.7 ± 0.3, $p < 0.05$ vs control for both). In contrast, the hydrophilic pravastatin (100 μM $5.6 \pm 1.0\%7$ p NS) failed to induce VSMC apoptosis. The effects of lipophilic statins were specific, as mevalonate (100 μM) fully reversed, in a dose-dependent manner, the statin-induced VSMC apoptosis. Cholesterol depletion was not responsible for these effects, as cholesterol was present in the serum-supplemented medium. To further evaluate which mevalonate metabolites might be involved in ATV-induced apoptosis, the ability of several mevalonate metabolites to reverse ATV effects was assessed next. Isopenteniladenine, ubiquinone (coenzyme Q10), and squalene failed to reverse ATV-induced apoptosis. In contrast, farnesyl-pyrophosphate (PP) (5 μM) and geranylgeranyl-PP (5 μM), in a dose-dependent manner, completely overrode ATV effects.

These data demonstrate that lipophilic HMG-CoA reductase inhibitors such as atorvastatin may induce apoptosis of VSMC even in the presence of the survival factors provided by serum. This effect is probably mediated by a depletion of cellular isoprenoids. Statin-induced apoptosis of VSMC may contribute to the control of neointimal thickening achieved by HMG-CoA reductase inhibitors in experimental models in the absence of changes in circulating lipids.

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Double-blind comparison of the quality of life of hypercholesterolemic patients treated with simvastatin or pravastatin

Seed, M, DM FRC Path, For The International Quality of Life Multicenter Group

Short Title: Quality of Life with Simvastatin and Pravastatin

Key Words: HMG CoA reductase inhibitors; Simvastatin; Pravastatin; Hypercholesterolemia; Randomized controlled trial; Quality of life; Efficacy; Safety

The efficacy, safety, and impact on quality of life of once daily treatment with simvastatin 20 mg and pravastatin 40 mg were compared in a multinational, randomized, double-blind trial involving 387 patients with primary mild to moderate hypercholesterolemia. The trial consisted of a 12-week baseline period, during the last 6 weeks of which patients received single-blind placebo, and a 12-week double-blind active treatment period. Throughout the trial, patients were maintained on a standard lipid-lowering diet. Treatment with simvastatin 20 mg/day for 12 weeks was associated with significantly greater reductions in plasma total and low density lipoprotein (LDL) cholesterol (25.7% and −33.6%, respectively) compared to pravastatin 40 mg (19.0% and −26.3%). By Week 12, nearly twice as many patients treated with simvastatin (48%) relative to pravastatin (26%) achieved target LDL cholesterol levels recommended by the National Cholesterol Education Program ATP II guidelines. The superior cholesterol lowering effects of simvastatin were not associated with any deleterious impact on health-related quality of life measurements. In the simvastatin group, mean changes after 12 weeks of treatment on each of the six areas evaluated in the self-administered health-related quality of life questionnaire, the Nottingham Health Profile (NHP), were negligible with only the evaluation of emotional reaction statistically significant relative to baseline scores. At least threequarters of simvastatin-treated patients indicated no change in response on NHP domains from baseline scores. Pravastatin had a similar negligible impact on health-related quality of life in this trial. The significant differences in the changes in lipid profiles between simvastatin 20 mg-and pravastatin 40 mg-treated groups did not result in any observed differences in tolerability and quality of life measures.

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The differential effects of pravastatin, lovastatin and simvastatin on second messenger pathways

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HMG-CoA reductase inhibitors now play a major role in the treatment of hypercholesterolemia. Although the specifity of these agents has been well established, little is known regarding the effect of HMG-CoA reductase inhibitors on the regulation of cell function. We previously demonstrated that lovastatin (L) and simvastatin (S), but not pravastatin (P) interfered with cell division and cholesterol metabolism in rat striated muscle cells (RTSM; (Mol Cell Cardiol 27, 2397–2402,1995) and on the expression of receptors, G-proteins and effectors which regulate the autonomic response in cultured heart cells (J Biol Chem 269, 30707–30712,1994). Calveolae have been shown to be the site of localization of receptors and G-proteins in the membrane. We compared the effect of P, S, and L on expression of G-α_{i2} and caveolin, a 20-KDa protein which is the principle protein component of caveolae, in RTSM and human umbilical vein endothelial cells (HUVEC). RTSM incubated overnight with 15 μM S or L demonstrated a 2-fold decrease in the levels of G-α_{i2} by Western blot analysis while 150 μM P had no effect on levels of G-α_{i2}. Incubation of RTSM with 15 μM S resulted in a 3 fold decrease in caveolin-3 while 15 μM L resulted in a 2-fold decrease in caveolin-3. Incubation of HUVEC with 10 μM S resulted in a 3 fold decrease in G-α_{i2} while 150 μM P had no effect on G-α_{i2}. Incubation of HUVEC cells with 15 μM S resulted in a 3 fold decrease in caveolin-1, while P had no effect. These data suggest that S and L may coordinately regulate caveolin and G-α_{i2} and may interfere with receptor effector-receptor coupling during the treatment of hypercholesterolemia. The differential effects of L, S and P in RTSM might be related to the decreased incidence of myositis in patients treated with P. Supported by a grant from Bristol Myers Squibb Company.

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Hepatic responses to inhibition of HMG-CoA reductase: a comparison of atorvastatin and simvastatin

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We have studied the cellular responses to simvastatin (S) and atorvastatin (A), two of the most potent HMG-CoA reductase inhibitors that have been used in humans. The two drugs exhibited similar IC_{50}'s for inhibition of HMG-CoA reductase from either rat or human, and single oral dosing in rats showed the compounds to be nearly equipotent at inhibiting the incorporation of labeled acetate into hepatic cholesterol. To study the adaptive responses to inhibition of reductase, induction of hepatic HMG-CoA reductase was studied after treating rats with S or A in the feed for four days. At each of two doses, both statins yielded comparable induction of HMG-CoA reductase activity and protein. For example, 0.05% S induced reductase activity 27.3 ± 9.1 fold and A induced activity 26.9 ± 4.7 fold. To examine the mechanism underlying this adaptive change, we turned to an in vitro system in which a steady state response to a fixed concentration of the statin could be achieved. HepG2, a human hepatocyte line, was cultured for 24 h in delipidated serum and then for an additional 24 h with graded doses of A or S. Over a broad range (10 nM to 10 μM), both drugs caused similar inductions of HMG-CoA reductase activity. Since cells regulate the level of reductase both through alterations in mRNA level and in degradation rate of the enzyme, we made parallel measurements of reductase protein (by western blot) and reductase mRNA (by RNA protection assays). S and A caused similar, dose-dependent induction of protein (~10-fold) and mRNA (~2-fold). If one of these two drugs had effects on either mRNA levels or reductase protein degradation that were not shared by the other drug, the ratio of mRNA/protein induction would differ. This ratio, however, was indistinguishable for S and A. To determine if A and S might yield differences in the subsequent responses to exogenous sterols, cells were treated with vastatin for 22 hr then challenged with 25-OH cholesterol for 2 hr. Cells responded with reductions in reductase mRNA and protein, and these changes were identical in cells treated with S or A. We conclude that these two drugs induce adaptive responses that are qualitatively and mechanistically identical.

We observed a slight kinetic difference in the inhibition of hepatic cholesterol synthesis after oral dosing in rats with the effects of S peaking before those of A. Studies of plasma half life in man have shown that A is cleared much more slowly than S. This pharmacokinetic difference, rather than some other difference in mechanism, is a likely explanation for the finding that the equipotent dose ratio for cholestcrol lowering in humans of S to A is about 2/1.

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Basal and TNF-Alpha stimulated production of PAI-1 by human adipose tissue in culture

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To verify if human adipose tissue can produce plasminogen activator inhibitor-1 (PAI-1), as recently demonstrated in mouse adipose tissue, subcutaneous and omental fat, obtained from surgical patients without malignant diseases, was cut in small fragments and incubated in medium 199 with Hank's salts either in basal conditions or in the presence of human recombinant TNF-alpha from 10 to 40 ng/ml. In basal condition, PAI-1 level (ELISA) in the incubation medium rose from 0.1 ng/ml after lh, to 1 ng/ml after 4hrs, 6 ng/ml after 8hrs, up to 40 ng/ml after 24hrs; the rate of PAI-1 production and release was therefore increased along with the incubation time. That effect of the incubation time in basal conditions was even more evident when the tissue samples were preincubated in basal medium for 24–48 hrs and then incubated for 48hrs in fresh basal medium. When TNF-alpha was present in the medium, increased PAI-1 levels were found in comparison with those observed at the respective culture periods in the absence of TNF-alpha. Such a TNF-alpha effect ranged between 130 and 300% of respective control conditions and was dose-dependent at the concentrations tested in this study. As expected, Northern blot analysis revealed the presence of mRNA of PAI-1 in those samples of adipose tissue. Furthermore, immunohistochemistry showed that both endothelial cells and adipocytes actively produce PAI-1. This study firstly demonstrates that human adipose tissue and the human adipocyte itself produce PAI-1 and that TNF-alpha, a cytokine physiologically present in adipocytes, can enhance that production. Moreover, the effect of the incubation period in basal conditions on PAI-1 release might suggest that the production of PAI-1 "in vivo" by adipose tissue is under the control of a inhibitor which is progressively removed during the culture.

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Plasma lipid peroxide levels and antioxidant enzyme activities in Type II diabetics: relationship with diabetic microangiopatic complications

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The relationship between glycosylated haemoglobin (HbA_{1c}), lipid peroxide (LPO), superoxide dismutase (SOD), glutathione peroxidase (GPx) with and without retinopathy in diabetes mellitus was investigated. The study included 57 patients with diabetes mellitus type II and 40 healthy controls. The control group and the group of type 11 diabetics subdivided to with and without retinopathy and evaluated for the following parameters. The mean values of HbA_{1c} and plasma LPO in diabetic patients were significantly higher than those in the control subjects ($p < 0.001$). Erythrocyte SOD activity of diabetic patients was found significantly lower compared with non diabetics ($p < 0.001$). There was no difference in erythrocyte GPx activity between the normals and the diabetics. We have observed that HbA_{1c} and plasma LPO levels were higher in diabetic individuals with retinopathy than those without ($p < 0.001$).
We concluded that increased LPO and decreased antioxidant defence syitem in diabetes mellitus may induce the onset of diabetic microangiopatic complications.

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Lysophosphatidylcholine in oxidized LDL may be involved in lipoprotein lipase down-regulation in human monocyte-derived macrophages

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Lysophosphatidylcholine (LysoPC) is an imporant lipid messenger, present in oxidized LDL and in β-VLDL. which has multiple biological effects on cell growth and macrophage proliferation. It specifically stimulates the activation of adenylyl cyclase in hurnan platelets and THP-1 cell line leading to production of cAMP. It also regulates the expression of several genes implicated in cell adhesion. In the present study, the role of oxidized LDL and of lysoPC on macrophage LPL activity and expression was examined. LDL obtained from plasma of normolipidemic blood donors were oxidized in vitro by incubation with SO_4Cu^{2+} (2.5 μM for 0.5 mg LDL protein/ml) during 2, 4, 6 and 48 h. OxLDLs (100 μg/ml) were added for 24 h to human monocyte-derived macrophages obtained after 12 days of culture. We have found that LDL oxidized for less than 6 hours, had no effect on LPL activity and mRNA levels. An inhibitory effect on LPL activity was detected with LDL oxidized for 6 h (33%, $p < 0.01$), which represents half of the inhibition observed with LDL oxidized for 48 h (60% for activity, 42% for mRNA levels, $p < 0.01$). The oxidation parameters of the OxLDL showed a progressive increase in relative electrophoretic mobility (REM), lipoperoxides (LPO) and thiobarbituric acid reactive substances (TBARS) levels. REM and LPO appeared to reach the same levels after 6 and 48 h oxidation. LDL lysoPC content increased also with time of oxidation whereas phosphatidylcholine decreased inversely during the same lag of time. In LDL oxidized during 6 h, lysoPC levels were about half of those found after 48 h of oxidation. LysoPC added in vitro to macrophages at concentrations of 1, 5, and 10 μg/ml (levels in the range found in LDL oxidized for 48 h), decreased LPL activity by 30%, 40% and 53% respectively, $p < 0.01$). It appears that lysoPC produces the same effect as extensively oxidized LDL and this LDL component could mediate the down-regulation of LPL expression in macrophages. We suggest that one regulatory mechanism triggered by lysoPC could imply intracellular cAMP synthesis, but its relationship with LPL synthesis and expression in macrophages needs to be defined.

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Effect of long-term ACE inhibition on oxidized LDL-induced endothelial dysfunction

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Oxidized (ox) LDL plays a key role in hypercholesterolemia-induced impairment of endothelium-derived (ED) NO-mediated responses. To assess whether long-term ACE inhibition may prevent this E dysfunction, 4 groups of rats were treated in parallel; group 1 (Ram 10) received ramipril 10 mg/kg/day p.o. for 6 weeks; group 2 (Ram + Hoe) received ramipril 10 mg/kg/day p.o. for 4 weeks and the last 2 weeks a cotreatment with ramipril plus HOE140 (a bradykinin B_2 antagonist 500 μg/kg/day, sub-cutaneous injections); group 3 (Ram 1) received ramipril 1 mg/kg/day p.o. for 6 weeks; group 4 served as control. Rings of thoracic aorta from these groups were contracted by phenylephrine (Phe) and relaxations to acetylcholine (Ach, 10 nM to 0.1 mM) were determined in the absence (oxLDL−) and in the presence (oxLDL+) of human Cu^{2+}-oxidized LDL (300 μg/ml, 30 min preincubation):

Groups		Maximal Response (%)		Area under curve
	0xLDL−	oxLDL+	oxLDL−	oxLDL+
Ram 10 (n = 9)	97 ± 2*	93 ± 5	358 ± 19*	369 ± 22
Ram+HOE (n = 9)	82 ± 3	48 ± 3+	284 ± 10	165 ± 9+
Ram 1 (n = 12)	91 ± 4*	76 ± 7+	289 ± 20	250 ± 32
Control (n = 12)	82 ± 4	53 ± 5+	269 ± 15	202 ± 17+

(mean ± SEM); % of Phe-induced plateau; * $P < 0.05$ vs control; +$P < 0.05$ vs oxLDL−.
The toxic effect of oxLDL is directly related to the NO synthase pathway as L-arginine (0.1 mM) coincubated with oxLDL completely prevented the E dysfunction in control group. Aortic cyclicGMP content, reflecting NO availability, was significantly increased in Ram 10 (by 76% vs control) but not in the other groups. Thus, long-term ACE inhibition dose-dependently protects against the acute toxicity of oxLDL; the mechanism is bradykinin-dependent and seems to involve an upregulation of EDNO synthase pathway via B_2 kinin receptor.

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The Animal Model in Atherosclerosis and Lipoproteins Investigation

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What are the differences between man and animals, and why is it that many animal species do not develop atherosclerosis? Is it because of the differences in the lipoprotein profile and lipid metabolism?
Animal species such as the dog, have lipoprotein profile significantly different from that of human. The major lipoproteins HDL-l and 2 fraction in the dog (and cow, sheep, goat and others) is the high density lipoprotein, (consisting about 60–80% of the total lipoprotein cholesterol) in comparison to humans having 20–35%. Can it be that this is the reason why atherosclerosis is rarely seen in the dog, even though the diet and living conditions are similar. Atherosrclerosis in dogs was reported only in hyperlipidemias associated hypolipoproteinemia.
The use of animal models in the investigation of lipids metabolism and its relationship to the development of atherosclerosis, could be very useful for the understanding of the pathogenicity, the sequence of events taking place and possible way to prevent it.
The models of dog, rat, cow, and chicken, having different nutritional and living environments, at different stages of life, and age and in relation to various pathogenic conditions will be described. The lipoprotein and lipid profiles in the sera of these animals will be detailed and compared.
Of interest is the cow's model, which serum lipoproteins levels change with age and the development of the rumen. Furthermore, the relationship between the lipoproteins and the development of fatty liver will be discussed. A direct relationship between the serum levels of high density lipoprotein and liver lipidosis was seen.
Differences in the lipoproteins characteristics (molecular weight, apolipoproteins size and aminoacids composition) are seen between humans and animals, as well as the absolute levels of the various lipid fractions (cholesterol, phospholipids and triglycerides).

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Effect of dietary amount and fat saturation on the regulation of apolipoprotein A-I mRNA and protein expressions in rats

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Low incidence of coronary ischaemic disease is associated with elevated levels of plasma high density lipoproteins (HDL). The main apolipoprotein constituent of these particles is apolipoprotein A-I (apo A-I). In previous studies, we have described an hepatic increase of mRNA for apolipoprotein A-I as consequence of feeding a monounsaturated- enriched fat diet. The present study has been designed to explore the relationships among this finding and plasma concentration of high density lipoproteins and apolipoprotein A-I. In the present study, we have also addressed the role of different amount of fat on the same parameters. To these goals, seven groups of male Wistar rats have been established: Control group consuming chow diet, Cholesterol group fed on a chow diet containing 0.1% (w/w) cholesterol, Coco group fed on a chow diet containing 0.1% (w/w) cholesterol and 40 % coconut oil, Corn group group fed on a chow diet containing 0.1% (w/w) cholesterol and 40 % corn oil and three Olive groups consuming a chow diet containing 0.1% (w/w) cholesterol and percentages of 5,10 and 40% respectively of olive oil. Animals were kept on these diets for two months and then sacrificed for lipoprotein, apolipoprotein and hepatic mRNA analysis. The main findings of this work are that the monounsaturated diet does not decrease plasma HDL cholesterol levels as does polyunsaturated fat diet. Both diets saturated and monounsaturated induced an increase in plasma concentration of apolipoprotein A-I. This increase is dependent on the ingested amount of fat in the later case. Considering the correlation between plasma apolipoprotein A-I concentration and hepatic mRNA expression in these diets, it seems that the plasmatic apo A-I increase in olive diets is mainly due to the hepatic mRNA expression. This fact is more relevant as the dietary amount of olive oil is increased. Nonetheless, the observed plasma apo A-I increase in saturated fat diet is not related to the hepatic mRNA content. All these data together are consistent with the hypothesis of that different fatty acids regulate apolipoprotein A-I expression through different mechanisms.

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A survey of total error in HDL-c measurements by chemical precipitation and homogeneous methods in 25 Dutch clinical labs

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As a result of the enhanced role of HDL-c in medical practice and the recently published US National Cholesterol Education Program (NCEP) guidelines for analytical performance of HDL-c assays, a pilot survey was conducted in the Netherlands, examining the analytical performance of conventional chemical precipitation methods and new homogeneous HDL-c assays. Laboratory inclusion was random, except for the type of HDL-c method. Accuracy was verified through a split-sample comparison with fresh human specimens. In parallel, the suitability of lyophilized candidate reference materials for HDL-c, containing various concentrations of saccharose and/or lipids, was examined. The point of reference was the CDC Designated Comparison Method, which was carried out in a CDC Network Laboratory. Routine HDLc analyses were done locally, by measuring six native specimens, in duplicate, during three consecutive days. Precision (CV_a) was calculated from measurement repeats. Total error was calculated as: 1.96 * CV_a (%) + absolute mean bias (%). HDL-c methods evaluated were: PEG (N = 7); PTA/$MgCl_2$ (N = 9); DS/$MgCl_2$ (N = 6); PEG/DS/$MgCl_2$ (N = 3); homogeneous HDL-c Boehringer (N = 3) and lyophilized N-geneousTM HDL-c Genzyme (N = 2).
In the chemical precipitation method group (N = 25), average (range) percent total error, bias and precision were 11.5% (2.7–5.2%), −0.65% (−14.2–± 11.5%) and 2.8% (0.8 − 8.0%) respectively. I.e., 24/25 labs met the ***interim*** total error goal of ≤ 22%. Moreover, 17/25 labs also satisfied the ***1998*** total error goal of ≤ 13%, the bias criterion of ≤ 5% being met in 12/25 labs, and the precision criterion of ≤ 4% being reached in 20/25 labs.
In the homogeneous HDL-c method group (N = 5), average (range) percent total error, bias and precision were 5.7% (3.3–8.4%), 1.5% (−1.9–± 4.6%) and 1.7% (0.7–2.6%) respectively, signifying that 5/5 labs were able to satisfy the ***1998*** performance goals.
It is concluded that the analytical performance of conventional chemical precipitation methods for HDL-c is sufficient to satisfy the ***interim*** NCEP total error goal in most of the clinical laboratories. To satisfy the ***1998*** total error goal, better standardization of chemical precipitation methods is a prerequisite. The new homogeneous methods for HDL-c seem to be reliable alternatives, that have ample potential to meet the ***1998*** NCEP goals.

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Modulation of lipoprotein(a) atherogenicity by serum HDL-c in middle-aged men with symptomatic coronary artery disease and moderately elevated serum cholesterol

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Conflicting reports have been published lately, confirming or refuting the association of lipoprotein(a) with clinical events or angiographically verified coronary artery disease (CAD). This study sought to examine whether lipoprotein(a) levels predict coronary artery lumen changes in patients with symptomatic coronary artery disease (CAD) and normal to moderate hyper-cholesterolaemia. Hitherto, the association between serum lipoprotein(a) and coronary artery lumen changes was studied in 704 males who entered REGRESS, a double-blind, placebo controlled, quantitative angiographical study which assessed the effect of two year of pravastatin treatment. The primary endpoints were change in average mean segment diameter (MSD) and change in average minimum obstruction diameter (MOD). Cases and controls were classified into progressors, regressors and stable patients, and median lipoprotein(a) concentrations were compared. Bi- and multivariate regression analyses were performed in the overall patient group and in high-risk subgroups.
In placebo treated patients median *in-trial* apolipoprotein(a) levels in regressing, stable and progressing patients were 130, 162 and 251 U/liter respectively, while 143, 224 and 306 U/liter in pravastatin treated patients. Predictors of MSD (MOD) changes were baseline MSD (MOD), *in-trial* apolipoprotein(a), *in-trial* HDL cholesterol and baseline use of long acting nitrates. The multivariate models explained 14% and 12% of respectively MSD and MOD changes; apolipoprotein(a) explained 2.6% and 4.8%. In patients with *in-trial* HDL cholesterol levels below 0.7 mmol/liter, apolipoprotein(a) correlated much stronger with the coronary score changes, explaining up to 37%.
It is concluded that serum lipoprotein(a) predicts coronary artery lumen changes in normal to moderately hypercholesterolaemic Caucasian males, its atherogenicity being especially daunting in case of concomitant hypoalphalipoproteinaemia.

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Heterogeneity of LDL receptor mutations in Dutch Familial Hypercholesterolemic patients.

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Introduction: Familial Hypercholesterolemia (FH) is a heterogeneous disorder at the molecular level. Over 300 mutations in the LDL receptor gene have been identified worldwide. However the spectrum of mutations occurring in different populations greatly varies. Knowledge of the spectrum of mutations causing FH in a given country is highly desirable, since it allows for molecular, and therefore unequivocal, diagnosis even in young patients in which the physical stigmata have not fully developed. LDL receptor mutation analysis was conducted in a large group of Dutch FH patients.
Patients: 153 patients were selected for the study, the vast majority which were children below the age of 16 years with a total cholesterol level over the 95th percentile for sex and age and a positive family history for premature cardiovascular disease.
Methods: The entire coding and splice-site consensus sequences of the LDL receptor gene were screened for sequence alterations. The nature of the sequence variation was then characterised by solid-phase direct sequencing.
Results: A sequence variation was detected in 112 patients (73%). In these, 47 different point mutations were identified, 16 of which were novel. Most of the mutations found are unique. Considered together with the 10 large rearrangements already characterised in Dutch FH patients, 57 mutations are responsible for FH in the Dutch population, explaining more than 50% of Dutch FH cases.
Conclusions: Identification of the majority of FH-causing mutations contributes to the quality of clinical diagnosis and facilitates large scale-screening.

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Influence of the ox-LDL on VEGF and iNOS gene expression in the experimental model of restenosis after PTCA.

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The endothelium-derived nitric oxide (NO) in addition to its potent vasorelaxatory and antiplatelet properties, inhibits growth of vascular smooth muscle cells (VSMC). Therefore the reendothelisation may be the limiting step for the formation of neointima. The expression of iNOS and vascular endothelial growth factor (VEGF) genes were studied by RT-PCR in the VSMC tissue culture after induction with IL-lβ (l–10 ng/ml) and in the baloon-induced denudation of the rat carotid artery model of angioplasty. Baloon-induced injury resulted in the iNOS expression in damaged but not in the sham-operated arteries, observed from 3 hours to 2 weeks. Expression of $VEGF_{120/121}$ isoform was found in control arteries when the additionally expression of the other $VEGF_{164/165}$ isoform was found in the operated arteries starting from 6 hours after surgery. Treatment of animals with L-arginine (2,25% solution in drinking water ad libitum starting from 3 days before surgery) decreased the neointima formation and intima/media ratio by 30–50%. In VSMC tissue culture IL-lβ induces both iNOS and the VEGF genes, however the induction of iNOS was observed within the first 2–4 hours of incubation, while the increase in expression of both isoforms $VEGF_{120/121}$ and $VEGF_{164/165}$ was observed later, 24 hours after addition of IL-lβ. The 24 hours long preincubation of rat VSMC with oxidized low density lipoprotein (ox-LDL) (human LDL incubated 24 hrs with 5μM Cu^{++} at temperature 37°C, 22 μM MDA), but not native LDL (0.5 μM MDA) completely abolished IL-β-induced iNOS and the VEGF genes expression 24 hours after IL- 1β addition.
We conclude that the angioplasty cause the induction of iNOS followed by the later induction of VEGF in the vessel wall. Since the both substances are deeply involved in the formation of neointima, the inhibitory effect of ox-LDL on the expresssion of genes of iNOS and VEGF argue for the necessity of the normalisation of the LDL blood level at the time of revascularization procedures of the man heart by the all available methods, including LDL-apheresis.

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Effects on garlic extracts on lipoprotein fractions and LDL oxidability

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Previous studies have suggested that pure fresh garlic or garlic extract may have beneficial effects on blood lipids and lipid oxidation. We investigated the effect of commercially available dried garlic powder tablets on these parameters.
Design. Twelve normal non obese men, 25 to 45 years of age were assigned to a balanced diet during three periods of 8 weeks. During the second 8 weeks period, they were given 3 x 2 tablets per day of SATIV'AIL®.
Results. Total cholesterol and triglycerides were reduced by 10.5% ($p < 0.0005$) and 15.5% ($p < 0.05$) respectively. In the LDL fraction separated by ultracentrifugation, there was a reduction of cholesterol (8.9%), triglycerides (20.7%) and protein content (13.7%) and a significant increase of the cholesterol/protein ratio (12.2%). In the HDL fraction, triglycerides and apoA-II were significantly reduced by 34.7% and 13.6% respectively, both in HDL2 and HDL3 subfractions. No significant change was noted in the VLDL fraction. LDL resistance to oxidation tended to increase (+7.9%, $p = 0.07$). In order to study the mechanism of action of garlic a second study was designed. Nine volunteers received tablets of SATIV'AIL® during a period of 36 days and plasma activities of several lipolytic enzymes were measured before and at the end of this period. Activities of lipoprotein lipase and lecithine acyltransferase increased respectively by 19% and 6%.
Conclusions: the quantitative and qualitative change in lipoprotein observed with garlic extract can be considered as beneficial for the prevention against atherosclerosis.
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In Vitro effect of lacidipine on induced low density lipoprotein oxidation

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The newly highly lipophilic 1,4 dihydropyridine lacidipine (L) was demonstrated to have a pronounced vascular protection effect, on both smooth muscle and endothelium. It is also able to reduce the formation of atheroma plaque in animal models at therapeutic doses. This protective activity might be explained by the antioxidant properties of L. The effect of L was therefore investigated on low density lipoproteins (LDL) oxidation initiated by two different oxidative systems: Cu 2+ and AAPH (2, 2 - azo bis (aminopropane) hydrochloride).
Design. LDL isolated by centrifugation from normocholesterolemic individuals were incubated with various concentrations of L. Oxidation of LDL was initiated by addition of CuCl2 or AAPH and monitored for production of conjugated dienes. The inhibition of production of conjugated dienes was studied by the change in the lag phase.
Results. For the CuCl2 experiments, the lag phase increased by 9%, 11%, 28%, 51% and 94% (5 experiments) with L at concentrations of 0.5, 1, 2.5, 5, and 10 μM respectively. For the AAPH experiments, the lag phase increased by 11%, 13%, 46%, 84% and 139% (4 experiments) with L at concentrations of 0.5, 1, 2.5, 5, and 10 μM respectively.
Conclusions. Lacidipine protects LDL from oxidative modification by copper and AAPH at a concentration at low as 1 μM. This concentration is far above therapeutic plasma levels, but due to its lipophilicity, it may be postulated that concentrations of L achieved in the LDL particles are much higher than those measured in plasma.
This work was supported By Boehringer Ingelheim.

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Moderate alcohol consumption in healthy volunteers reduced plasma clearance of apolipoprotein A-II.

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Mechanisms of the positive relationship between alcohol intake and plasma concentration of HDL are still unclear. The present study shows the modifications of the metabolism of apoA-I and apoA-II using an endogenous labeling with cold isotope in normolipidemic healthy volunteers after a period of moderate alcohol consumption.
Patients and Methods: Five non smoking male subjects were studied at the end of 2 consecutive 4-week periods, one without alcohol, the other with an intake of 50 $g.d^{-1}$ of alcohol, in a random order. The metabolic parameters of the apoA-I and apoA-II in HDL were determined after endogenous labeling using amino acid labeled with stable isotope.
Results: Cholesterol, triacylglycerols, HDL-cholesterol, apoA-I, apoA-II, LpA-I, LpA-I:A-II were dosed in serum at the end of the 2 study periods. Cholesterol and triacylglycerols did not vary significantly during the 2 periods whereas HDLcholesterol increased from 43.8 to 50.0 $mg.dL^{-1}$ ($P < 0.05$). ApoA-I and apoA-II increased significantly (20 and 60% respectively) after the diet was supplemented with alcohol. LpA-I:A-II increased from 73.8 to 101.6 $mg.dL^{-1}$ (+ 32%) ($P < 0.05$) while alcohol had no effect on the concentration of LpA-I. The alcohol treatment did not significantly alter the metabolism of apoA-I. Conversely, the fractional catabolic rate of apoA-II decreased significantly by 21% ($P < 0.05$) with alcohol while the production rate of apoA-II tended to increase by 18% ($p = 0.08$).
Conclusions: The decrease in the FCR of apoA-II could lead to the accumulation of apoA-II-containing lipoproteins in plasma and explain the dramatic increase in LpAI:A-II observed in the plasma of subjects consuming alcohol. However LpA-I did not vary significantly.

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Hypochlorous Acid and Human Blood Low Density Lipoproteins Modified by Hypochlorous Acid Increase Erythrocyte Adhesion to Endothelial Cells

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Oxidative modification of blood lipoproteins (LP) is known to be an important risk factor in the development of diverse diseases, such as atherosclerosis, diabetis, etc. One of the origins of oxidatively modified LP in blood is the interaction of native LP with active oxygen species generated by activated neutrophils and monocytemacrophages, among them hypochlorous acid (HOCl) or its dissociated form OCl^- (hypochlorite anion). Erythrocyte adhesion plays a major role in blood flow, particulary in the microcirculation, and its appearance is a very important stage in the development of pathological process. Oxidized low density LPs (LDL) are known to increase adhesion of blood cells to endothelium. It is the purpose of the present study to clear up whether HOCl/OCl^- or HOCl/OCl^--modified LDL promotes the adhesion of human erythrocytes to endothelial cells (ECs). The effect of preincubation of the ECs with different HOCl concentration or HOCl-modified LDL on adhesion was investigated. Human LDL were modified within 1 h incubation with the HOCl concentrations 0.1; 0.2; 0.5 and 1.0 mM. A rise in the HOCl concentration in the incubation medium led (i) to a significant decrease of LDL fluorescence towards the UV region (extinction by 285 nm, emission by 340 nm), (ii) to the accumulation of the secondary aldehyde lipid peroxidation products, (iii) to an increase of final (Schiff bases) products of lipid peroxidation, and (iiii) to the elevation of electrophoretic mobility of LDL. This gives evidence for free radical induced damage of proteins as well as of lipids, which resulted in an increased LDL negative surface charge. It was shown, that preincubation of ECs with HOCl (15 min, at 37°C, in 5% CO_2) at concentrations up to 50 μM leads to an increase of erythrocyte adhesion. The same effect was observed after the preincubation of ECs with HOCl-modified LDL. An increase in the HOCl concentration up to 250 μM, used during preliminary modification of LDL, led an increased cell adhesion. The increase of adhesion was in parallel to the increase of the molar cholesterol/phospholipid ratio in endothelial cells. Using HOCl concentrations above 50 μM or strongly modified LDL (preincubated with 500 μM HOCl), respectively, caused cytotoxic effects on ECs and led to only slight erythrocytes adhesion and cholesterol accumulation in endothelial cells. The results obtained suggest that HOCl in physiological concentration is able either directly or indirectly, due to LDL modification, to modulate the adhesion of erythrocytes to endothelium and cholesterol accumulation in vessel wall. Hence, it may play an important role in the development of atherosclerosis.

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A New Method to Investigate the Correlation of Initial Lesions of the Wall and the Blood Flow

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The influence of bloodflow on the deposits on the vessel wall has been observed and described already in 1856 by the pathologist Rudolf Virchow. There is an abundance of data connecting certain forms of bloodflow — separated and stagnant bloodflow — to alterations in the vessel wall. However, the exact correlation is not yet known, especially it is not known, how the alteration is initiated. There is no known method, how one can detect initial lesions — fatty streaks — of the wall in vivo and to connect it to the bloodflow. However, fatty streaks can be easily detected post mortem. Therefore the method presented here is based on the post mortem assessment of the initial lesions. The bloodflow — no longer existing in the cadaver — is subsequently reconstructed in a fluid mechanical model. The carotid arteries are excised from the cadaver, mounted in an apparatus especially designed for making vessel casts. The vessels are pressurized to 80 mmHg and filled with a vessel cast polymer. After the polymer is hardened, the artery is opened dorsally, attached to a wet porous cardboard and is placed into a solution of 10% neutral formaldehyde for 72 hours. The artery is then removed from the cardboard and put into a vessel with 10% neutral formaldehyde solution. Now the artery is ready to be analyzed pathomorphologically and morphometrically. The methods follow rules described 1958 by the WHO group of experts on atherosclerosis (Atherometric System). The locations of pathomorphological alterations of the wall, i.e. fatty streak, fibrous plaque and advanced lesions are defined and assessed. The vessel casts are used for the fabrication of flow models. To achieve this, the vessel casts are submersed in a liquid transparent silicone rubber. Vacuum is used to remove air bubbles in order to achieve a good optical quality. After curing, the silicone rubber mold is cut open and the vessel cast is removed. The silicone mold now represents an exact replica of the lumen of the original vessel. This replica is placed in a flow duplicator especially designed for the purpose. The flow is pulsatile and the partition of the flow at the bifurcation is volumetrically controlled. Particles are added to the fluid to visualize the flow. The visual images of the flow are processed and deliver the shear rate at the wall. The wall's shear rate reflects all disturbances of the flow, such as, flow separations and stagnation lines. The result is a method, which permits a comparison of the individual lesions and anatomical shape of the vessel with the flow, as it existed during the lifetime of the deceased individual.

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Automated enhanced chemiluminescence technique in evaluating total antioxidativ capacity in human serum

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Oxidative stress has been discussed in the pathogenesis of a number of diseases and oxidation of lipoproteins has been suggested to be an important factor in the atherosclerotic process. A number of antioxidants prevent oxidative reactions and a decreased level of antioxidant status may reflect human disease. Enhanced chemiluminescence technique has previously been described by Whitehead et al in measuring total antioxidant capacity (TAOC) in human serum. We have further developed this method to a complete automised computer assisted luminometric system where the duration of light suppression is related to the amount of antioxidant present in each serum sample. Trolox is used as a standard antioxidant solution. Adding trolox to different serum dilutions gave a parallell shift of the curve corresponding to the concentration of added trolox. Coefficient of variance regarding the reproducibility of our method was 6%. TAOC-values in fresh serum samples from 10 individuals ranged between 230-410 mmol trolox equivalents/litre. Serum stored in −80° C for up to 6 months showed no major effect on the TAOC-value but after 12 months storage a decrease in the TAOC-value of 15% was noticed. To correlate TAOC with a method measuring 7 individual antioxidative parameters plus uric acid, measurements were performed in 12 healthy individuals. Regression analysis gave a total correlation between the measurments of the individual antioxidants and the TAOC measurment of 99.7%. The effect of red wine intake on TAOC and uric acid was measured in 15 healthy individuals. Measurements were performed both after water and after red wine intake in the same individuals. TAOC increased with 23 percent 30 min and 90 min after intake of red wine compared to water intake and corresponding values for uric acid was 30 and 25 percent. In conclusion, automised enhanced chemiluminescence technique may give a rapid, safe and simple way to analyse total antioxidative capacity.

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Is the measurement of unbound apo(a) of diagnostic interest?

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The various methods for quantitation of Lp(a) occasionally give significantly different results. The basis for this discrepancy is not fully understood yet. In our view a great part of differences in Lp(a) concentrations between different Lp(a) methods are caused in this way that apo(a) partly circulates in plasma unbound (not bound to apo B). There are methods which measure free apo(a) plus intact Lp(a) while other methods only determine intact Lp(a).
In this study we have measured the Lp(a) level in serum using three commercial test systems and compared the results with a novel technique that determines unbound apo(a): A) nephelometric Lp(a) test (Behring, Germany), B) sandwich ELISA (Cardio Check) with fixed anti-apo(a) capturing Lp(a) and with enzyme labelled anti-apo B for detection (USA), C) sandwich ELISA (Immunozym) with fixed anti-apo(a) capturing Lp(a) and with enzyme labelled anti-apo(a) for detection (Immuno GMBH, Germany), D) sandwich ELISA for unbound apo(a) (Immuno GMBH, Germany). Our study included 140 coronary patients and healthy subjects.
The Lp(a) results of the nephelometric test were in good agreement with the results from ELISA Immunozym [anti-apo(a) for capture as well as for detection] while significant lower Lp(a) concentrations with the ELISA Cardio Check especially in higher Lp(a) concentration range were determined. The ELISA Cardio Check is specific only for intact Lp(a) because an enzyme labelled apo B antibody is used within the assay. The (non-linear) increase of the concentration of unbound apo(a) correlates with a linear increase of the Lp(a) concentration resulting from test systems which measure intact Lp(a) plus unbound apo(a) (nephelometric test, ELISA Immunozym). The ascent of the linear Lp(a) curve which represents intact Lp(a) plus unbound apo(a) is much steeper compared to the ascent from the Lp(a) curve representing only intact Lp(a). The increase of Lp(a) in the concentration range above 50 mg/dl is due to a very strong increase of unbound apo(a).
Unbound apo(a) probably is an interesting parameter for risk assessment in patients with coronary heart disease. Our results clearly show that an increased atherogenic risk by high Lp(a) level is mainly due to an increase of unbound apo(a).

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The importance of hyperhomocysteinemia in high age people

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A disturbed methionine metabolism with moderate elevated homocysteine level has been recognised as an independent atherogenic risk factor. Elevated plasma homocysteine is found in 20–50% of patients with premature CHD, stroke, or peripheral vascular occlusive disease. Homocysteine also has great importance in geriatrics. Here is homocysteine an indicator for an intracellular lack of B-vitamins and/ or folic acid.
Therefore we investigated 70 healthy high age people (85–99 years) compared to 75 healthy seniors (age 65–75 years) for serum homocysteine levels. Homocysteine was investigated by HPLC with fluorescence detector. 65% of the high age people had elevated homocysteine level contrary to 40% of the healthy seniors (65–75 years). The healthy seniors only showed moderate homocysteine elevations (>15 to <30 µmol/l) while in the high group intermediate homocysteine elevations (>30 to <100 µmol/l) occurred in 17% of the subjects.
B- vitamins were decreased in 68% of high age subjects and in 30% of healthy seniors. The deficiency of vitamin B6 was in the high age group at 58% most frequent compared to the younger seniors at 17%. Vitamin B12 deficiency occurred in high age subjects at 25% and in younger seniors at 13%. Decreased folic acid was found in 3% in both age groups.
So the measurement of metabolites like homocysteine tells us more about intracellular vitamin B deficiency than the direct measurement of B-vitamins and folic acid. The very high prevalence of hyperhomocysteinemia in elderly is a strong indicator for an acute intracellular deficiency of B-vitamins and folic acid.
Beside vitamin shortage a very common mutation in the tetrahydrofolic acid circle, the mutation of the thermolabile methylene tetrahydrofolic acid reductase (MTHFR), is seen responsible for moderate homocysteine elevation. The 677-missense mutation in the MTHFR gene is found in 25% of allels in high age people and in 35% in of allels in controls. So the MTHFR gene mutation is less frequent in subjects of high age than in younger subjects.

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Evaluation of two assays for the direct determination of HDL and LDL cholesterol

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HDL- and LDL-cholesterol are among the most important diagnostic indicators in the assessment of coronary heard disease risk. The measurement of HDL-c and LDL-c required pre-treatment of the sample which is time consuming and also affecting accuracy and reproducibility. Two new assays are from Boehringer Mannheim allow the direct determination from primary tubes. The HDL-c assay principle is based on the specific measurement of HDL cholesterol with an enzymatic procedure after masking of the LDL and VLDL lipoproteins. LDL-c is measured by a turbidimetric reaction caused by the specific agglutination of LDL lipoproteins. The HDL-c and LDL-c assays were standardized to phosphotungstic acid precipitation and the Beta-quant procedure, respectively. The new assays were evaluated in 16 laboratories in Europe, Japan and the USA. The total imprecision of both assays determined in 21 analytical runs was below 4 CV % in the normal and pathological concentration range (median results). Similar CV's were obtained with two quality control samples measured each analytical run, and the mean recovery was in the range of 94–115% (HDL-c) and 96–105% (LDLc). The new assays were compared to routine procedures including precipitation after ultracentrifugation performed at some evaluation sites. The direct HDL assay correlated well to phosphotungstic acid precipitation (r: 0.957–0.992) and the CDC reference method (heparin/Mn precipitation after ultracentrifugation, r: 0.994). The correlation of the direct LDL-c assay to routine assays was in a range of r: 0.814–0.964 in comparisons to beta quantification, dextran sulfate and polyvinyl sulfate precipitation, electrophoresis, and an assay based on immuno-affinity separation of LDL lipoproteins. No interference was found in hemolytic patient samples with both direct assays. Bilirubin did not interfere with HDL-c and LDL-C determinations below 18 and 50 mg/dl, respectively. Lipemia did not affect the measuring results in native samples below triglyceride concentrations of 1200 and 400 mg/dl. Compared to routine procedures both direct assays substantially reduced the effort for HDL-c and LDL-c determinations in the laboratories while showing comparable analytical performance.

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APOB-Bound lipids as a parameter reflecting the level of LDL oxidation

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At present, it is commonly recognized that oxidatively modified LDL plays an important role in atherogenesis. However, determination of the degree of LDL oxidation *in vivo* is complicated due to a number of various lipoperoxidation products with the short life time as well as their loss during LDL isolation. In this work we studied the possibility of measuring the degree of LDL oxidation by determination of lipids covalently bound to apolipoprotein B. To demonstrate the existence of adducts of lipids with apo-B, LDL was oxidized by copper ions, 2,2'-azobis-(2-amidinopropane hydrochloride) as well as by macrophages. After the oxidation, LDL was delipidated by repeated extraction with isopropanol, chloroform-methanol mixture and diethyl ether. Accumulation of protein-bound sterols in oxidized LDL was demonstrated by Chugaev's reaction and high performance liquid chromatography after 30 min alkaline hydrolysis at 100°C. Presence of protein-bound phospholipids in oxidized LDL was registered by nuclear magnetic resonance technique as well as by calorimetric phosphate determination. Kinetic study has shown that in contrast to conjugated dienes, TBA-reactive substances and hydroperoxides, the content of protein-bound lipids was increasing monotonously up to 36 h of oxidation. Obtained data suggest that the content of protein-bound lipids is a parameter reflecting the degree of LDL oxidation and can be used to evaluate the role of lipoprotein oxidation in atherogenesis.The level of apoB-bound cholesterol in patients with coronary atherosclerosis was significantly higher as compared to healthy subjects. The ability of LDL to induce cholesterol accumulation in macrophage cell culture was independent from protein-bound cholesterol content.

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Peculiarities of HDL phospholipid composition in men with lower limb amputation.

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Epidemiologic studies have shown a strong inverse relation between HDL-c and the risk coronary artery disease (CAD). The protective effect of HDL is related to reverse cholesterol transport. HDL phospholipids (PL) determine the structure of surface monolayer and antiatherogenic properties of HDL, because PL play crucial role in some steps of this process: efflux of C from cell membranes, plasma cholesterol esterification, interaction with hepatic cells.
The men with disability following lower limb amputation have lipoprotein profile abnormalities as compared to men from Moscow male population. They show, in particular, lower level of HDL-C (44 ± 1.1 vs 52 ± 0.6 mg/dl, $p < 0.001$).
The objective was to carry out a comparative study of HDL phospholipid composition in amputees and healthy subjects. 102 men aged 17–59 with lower limb amputation and age-matched healthy men from Moscow population were included in this study. Lower level of HDL-C in amputees was associated with peculiarities in HDL phospholipid composition. Subjects, who had amputation as a result of obstructive arterial disease (aged 35–59, $n = 27$) had in HDL lower portion of phosphatidylcholine (PC, $66.8 \pm 1.4\%$ vs $70.8 \pm 0.5\%$) and phosphatidylethanolamine (PE, $3.6 \pm 0.1\%$ vs $4.1 \pm 0.2\%$), whereas portion of lysophosphatidylcholine (LPC) was higher ($16.6 \pm 1.5\%$ vs $11.5 \pm 0.4\%$). These subjects did not differ by content of sphingomyelin (SM) and cardiolipin (CL).
Older men (aged 40–59, $n = 17$) and younger men (aged 17–39, $n = 58$) with posttraumatic lower limb amputation as compared to age-matched healthy subjects had lower portion of PC ($66.9 \pm 1.4\%$ vs $74.9 \pm 0.5\%$ and $67.3 \pm 0.6\%$ vs $71.5 \pm 0.5\%$, respectively) and higher portion of LPC ($16.4 \pm 1.0\%$ vs $10.4 \pm 0.4\%$ and $15.8 \pm 0.4\%$ vs $12.9 \pm 0.5\%$) and CL ($1.6 \pm 0.2\%$ vs $0.5 \pm 0.1\%$ and $1.8 \pm 0.2\%$ vs $1.1 \pm 0.1\%$), while portion of SM and PE did not differ.
Thus, obtained in amputees lower level of HDL-c and lower portion of phosphatidylcholine — major HDL phospholipid, which is involved in reverse cholesterol transport, can promote CAD development, especially, in younger amputees.

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Ultastructure-functional state of organs in experimental atherosclerosis

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The disorders of microcirculation state of various organs is known, but only a few study is concerned of the changes of organ(playing an important role in pathogenesis and morbidity and mortality)ultrastructure-functional state in atherosclerosis. In other hand, atherosclerosis begins from the early ages, develops "silently" and reveals in the complicated stage. The examination of organs presents a great difficulty in/vivo. That's way for the study of organ disorders from early stages of atherosclerosis we were used a rabbit atherosclerotic model. The experiments were carried out on rabbits in which experimental atherosclerosis was produced (male rabbits weighing 2.5–3.0 kg, received a cholesterol -rich diet 0.3 g by kg weight) for study the ultrastructural-functional changes of the different organs (heart, liver,lung, kidney, brain, aorta) in the various stages of the development atherosclerosis(2 week, 1, 2, 3, 4 and 6 month) .The animals were killed with i/v injection of 30 mg Thiopental sodium and the samples of organs after fixation and with corresponding treatment were cut on a Reichert OmU2-ultramicrotome. Sections were stained and examined in a Tesla BS-500electron microscope . The significant changes as in microcirculation (capillaries, postcapillaries venules wall ultrastructure) as in connective tissue and parenchyma cells in different degree are observed in various organs and stages of development of atherosclerosis. The hypothesis is proposed, that cholesterol diet provokes (by metabolic, toxic and anoxic influences) a replay from the cells generally and especially endotheliocyts as a connective tissue cells, responsible the structural-functional reconstruction of above mentioned vital importance organs .This suggestion may be used as basic mechanism for explication of developing the various clinical reveals even in early stage of human atherosclerosis.

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Hypochlorite-modified low-density lipoprotein stimulates human polymorphonuclear leukocytes for enhanced production of reactive oxygen metabolites

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Hypochlorous acid (HOCl) is a potent non-metal-dependent oxidant which is formed by myeloperoxidase (MPO, EC 1.11.1.7) from H_2O_2 and chloride ions. Recent studies have demonstrated that the exposure of isolated human LDL to hypochlorite oxidizes the lipoprotein efficiently, producing an aggregated high-uptake form for macrophages. Since polymorphonuclear leukocytes (PMNLs), which are contain exceptionally high concentrations of MPO have been found in the earliest atherosclerotic lesions they may serve as a potential source of HOCl/OCl^-- mediated LDL oxidation. Furthermore, it may reasonably be argued that PMNLs could serve also as a target of oxidized LDL. To investigate the potential stimulatory effects of HOCl-modified LDL on human PMNLs, we measured the intracellular and extracellular generation of reactive oxygen species (ROS) of NaOCl-LDL-stimulated PMNLs by means of chemiluminescence and the adherence of these PMNLs on human umbilical venous endothelial cells (HUVECs). We report here that OCl^--LDL but not native or Cu^{2+}-oxidized LDL stimulated the respiratory burst in a concentration- and time-dependent manner. About 70% of the generated ROS (H_2O_2 and superoxide anion) were released into the extracellular environment. The ROS generating capacity was diminished by cytochalasin B, an inhibitor of phagocytosis. Adherence of OCl^--LDL-stimulated PMNLs to HUVECs was increased 2–3 fold as compared to PMNLs treated with native LDL. The results indicate a potential role of PMNLs in initiating and/or maintaining the inflammatory process during the early phase of atherosclerotic lesion development by enhancing extracellular ROS generation and adherence to endothelial cells. Alternatively, PMNLs may also play a protective role by phagocytosing oxidized LDL and, thus, preventing from further detrimental atherogenic effects of oxidized LDL.

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Novel mutations in the LDL receptor gene of Dutch Familial Hypercholesterolemic patients.

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Knowledge of the spectrum of mutations occurring in a given population is becoming increasingly important for the implementation of molecular diagnosis of Familial Hypercholesterolemia (FH).
During the course of screening for mutations in the LDL receptor gene of Dutch FH patients 14 new mutations were characterised by Denaturing Gradient Gel Electrophoresis (DGGE) and solid-phase direct sequencing.
We identified 7 missense (C113R, K273E, G314V, C331W, C317G, D321N, P542H), 3 splicing (G→T2389, G→T2389+1, G→A2140+5), 2 deletions of 12 and 5 base pairs in exons 14 and 16 respectively, a duplication of 18 base pairs in exon 17 and 1 silent (P320P) mutation.
Surprisingly, in two unrelated patients three mutations (C317G, P320P and D321N), occurring in exon 7, a few bases apart from each other, were located on the same allele.
The mutations found are most likely associated with FH since these were the only molecular defects identified in the entire coding and splice-site consensus sequences. In addition, in most cases the functional consequences of the presence of these alterations on the LDL receptor protein activity can be predicted by structure-function anlysis. However, for those mutations for which predictions cannot be made, typically the G→A2140+5 mutation, functional studies are required to establish their pathogenicity.

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Acute Effects of Heparin on Triglyceride —, Apolipoprotein E — and Lipoprotein Lipase Levels in Humans.

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Apolipoprotein E (apoE) and lipoprotein lipase (LPL) play an important role in the metabolism of triglyceride (TG) rich lipoproteins. Both proteins share the ability to interact with proteoglycans (PG) and lipoprotein receptors and thus mediate the catabolism of TG rich lipoproteins. In vivo LPL is released from PGs on the endothelium after heparin injection. Separation of postheparin plasma lipoproteins by FPLC, followed by ELISA detection of lipoprotein lipase revealed LPL associated with TG-rich lipoproteins. It is not known whether apoE is released in a similar way by heparin in vivo. However, in vitro apoE can be released into the culture medium from human hepatoma cells by heparin treatment, suggesting the existence of a pool of PG bound apoE. We therefore studied the effect of intravenous heparin injection in 6 normal controls (3 with an apoE3/3 phenotype, 3 with apoE2/2) and 6 patients with mixed hyperlipidemia (3 with apoE3/3, 3 with apoE2/2). Total triglyceride and apoE mass, as well as LPL activity and mass were measured before, 10, 30, and 60 minutes after injection (p.i.) of heparin.
As expected an increase of LPL activity and mass was observed 10 minutes p.i. in all study subjects. TG and apoE levels showed a slight decrease at 10 min p.i, which continued over time reaching a > 50% decrease of TGs and apoE levels at 60 minutes, and a 40% decrease of LPL compared to 10 min. No significant difference between patients and controls and carriers of the e2 and e3 alleles was observed.
In summary: Injection of heparin results in a dramatic increase in LPL at 10 min. p.i, but almost unchanged TG and apoE levels. Over 60 minutes a continous decrease of plasma TGs, apoE and LPL reaching 60% in normolipemic control and hyperlipemic patients was observed. No difference between carriers of apoE2 and apoE3 could be shown. Therefore in contrast to LPL, no heparin releasable pool of apoE could be detected, though both apoE and LPL are able to bind to PGs.
We suggest that this effect of heparin on TG rich lipoproteins is due to an association of LPL with TG rich lipoproteins following the initial release of LPL from endothelial PGs. TG rich lipoproteins which have aquired LPL as a ligand will then be cleared by an increased cellular uptake as demonstrated by the parallell disappearance of TGs, apoE and LPL from the circulation.
These results support the role of LPL in mediating the catabolism of TG-rich lipoproteins due to its function as a receptor ligand, in addition to its role as lipolytic enzyme.

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Multicenter evaluation of a homogeneous assay for HDL cholesterol

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We evaluated a new homogeneous assay for the measurement of high density lipoprotein cholesterol (HDL-C) in six European laboratories. The assay includes two reagents and is applicable to most autoanalyzers which allows full automatization.
The total CV's of the new method ranged between 1.3 and 6.7%. Thereby determined HDL-C values were in good agreement with those determined after precipitation with phosphotungstic acid/$MgCl_2$ and those determined by the combination of ultracentrifugation and precipitation (r = 0.956–0.994). The assay was linear up to at least 1,700 mg/L HDL-C.
Lipemia up to total triglyceride levels of 8000 mg/L did not interfere with the homogeneous HDL-C assay. Intralipid® at triglyceride concentrations up to 50 g/L decreased the results by less than 10 %. Hemoglobin did not interfere, whereas icteric samples with bilirubin above 100 mg/L showed non systematic discrepancies between the homogeneous and the precipitation assay.
The homogeneous HDL-C assay was easy to handle and produced similar results in all laboratories participating in this study. This test will significantly facilitate the screening of individuals at increased risk for cardiovascular disease.

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Correlation between serum homocysteine and uric acid is influenced by MTHFR genotype

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A common mutation at nucleotide 677 (C-to-T substitution, Ala-to-Val) of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene results in enzyme thermolability and decreased activity and in elevation of homocysteine levels, which are a known risk factor for vascular disease. We studied an Italian population of 155 healthy individuals with a mean age of 38 years, in which biochemical and hematological parameters as well as biometrical and life style characteristics were investigated. The frequency of the genotype "+/+" (homozygosity for the rare allele) at the MTHFR locus was 16.1%. Homocysteine levels resulted to be significantly higher in men with genotype "+/+" but not in women. No difference between genotypes was evident in individuals with plasma folate or vitamin B_{12} levels at or above the median values. Homocysteine levels (adjusted for folate and vitamin B_{12}) were correlated to age, blood pressure, body mass index and alcohol intake. Furthermore, a correlation with known risk factors for coronary heart disease (total cholesterol, triglycerides, uric acid) was evidenced. Multiple stepwise regression analysis showed that uric acid was the only biochemical parameter predictive of homocysteine values ($P = 0.00001$) . Among the non biochemical variables, diastolic blood pressure and alcohol intake resulted to be highly predictive. Subgroup analysis according to the MTHFR genotype in men evidenced that these associations were restricted to the carriers of the rare allele. Thus, the MTHFR genotype seems to influence the correlation between homocysteine and other risk factors for cardiovascular disease.

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Lipoprotein composition in human prenodal calf lymph

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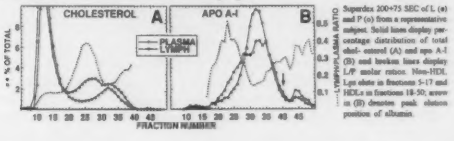
Most work on reverse cholesterol transport (RCT) has utilised cultured cells and plasma-derived lipoproteins (Lps). To learn more about RCT in human tissue fluids *in vivo*, prenodal lymph (L) was collected from a vessel in the medial aspect of the calf. This technique is more reliable, gives greater flow rates and permits longer collections than cannulation in the foot (Reichl/Pflug method). Studies were carried out in 11 healthy males aged 19–50 yrs after a light fat-free breakfast. L was collected into EDTA (lmg/mL) for 5 or 6h at flow rates of 0.5–3.5 mL/h. EDTA plasma (P) and L from the same subject were analysed together. Mean L/P ratios of unesterified (UC) and esterified cholesterol (EC), choline-containing phospholipids (PL), sphingomyelin (SPM), and apolipoproteins (apos) A-I, A-II, B and C-III were: 0.12, 0.12, 0.13, 0.14, 0.20, 0.19, 0.07,and 0.16. There were no significant differences in UC/EC,SPM/PL or apo A-I/apo A-II ratios between L and P, but the apo A-I/apo B ratio was higher in L (5.00 vs 1.69) and the apo B/apo C-III ratio was higher in P (10.90 vs 5.64). The L/P ratios of several non-Lp proteins (α_1-acid glycoprotein, 0.42; α_1-antitrypsin, 0.36; albumin, 0.34; transferrin, 0.35; IgG, 0.21; complement C3, 0.19; and α_2-macroglobulin, 0.09) were inversely related to MW; the L/P ratios of total apo A-I and total apo B were close to those predicted by the average MWs of high-density- (HDLs) and low-density- (LDLs) lipoproteins. By Superose 6 size exclusion chromatography (SEC) the concentrations of HDL UC, HDL EC and HDL PL in L were positively correlated with those in P ($p < 0.005$), but there were no significant correlations for apos, non-HDL lipids or non-Lp proteins between the two matrices. SEC using Superdex 200+75 in tandem separated L HDLs into a major population of 7.5–12 nm diameter particles and a minor one of 5.8–6.3 nm particles (Panel B, fractions 18–41 and 42–50, respectively). The proportion of total apo A-I in the small LpA-Is averaged 4.4% in L and 1.7% in P. After incubation at 37°C for 18h, the proportion increased in both matrices (to 8.7% and 3.5%); similar increases in total pre-β LpA-I mass were observed by anti-apo A-I crossed immunoelectrophoresis. In L relatively more cholesterol and apo A-I eluted with vely large HDL species (Panel A, fractions 20–30). The UC/CE ratios in these fractions of L were up to 2-fold greater than the UC/CE ratios in corresponding fractions of P. They were also relatively deficient in apo A-II, and co-eluted with a peak of apo E. On preparative agarose gel electrophoresis, 94.3–97.2% of L apo A-I migrated with α-mobility and 2.8–5.7% with pre-β mobility; apo A-II was present only in α-particles. Both apos migrated more rapidly in L than in P, indicating a greater negative surface charge. The electrophoretic mobility of apo B (β-migrating) was the same in L and P.
We conclude that considerable remodelling of HDLs occurs in the extravascular space *in vivo* as a consequence of uptake of cell-derived UC. The rate of transport of UC from cells exceeds its rate of esterification in tissue fluid. Pre-β LpA-Is can be generated extravascularly by a mechanism that is independent of cells. Advanced forms of oxidised LDLs appear not to enter blood from tissues via lymph.



Superdex 200+75 SEC of L (●) and P (○) from a representative subject. Solid lines display percentage distribution of total chol- esterol (A) and apo A-I (B) and broken lines display L/P molar ratios. Non-HDL Lps elute in fractions 5–17 and HDLs in fractions 18–50; arrow in (B) denotes peak elution position of albumin.

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Accelerated Cholesterol Synthesis in Monocytes of Individuals with Premature Atherosclerosis

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The rate of de novo cholesterol synthesis was determined in monocytes isolated and stored in a lipoprotein-free medium and then incubated for three hours with tritiated water. Synthesis was calculated in the absolute amount of incorporated label into isolated cholesterol. A group of 23 myocardial infarction male survivors under 50 years of age (5-24 months after MI) was compared with age and weight matched controls free of coronary heart disease and without a family history of premature atherosclerosis. Significantly higher LDL cholesterol and lower HDL cholesterol concentrations were found in the patient group compared to controls.
Cholesterol synthesis of MI survivors was doubled (0.82 ± 0.43 pmol/10^6 cells/hr) compared to controls (0.32 ± 0.08). In contrast to a low biological variation in the control group a very high variation in MI survivors made simple mathematical analysis impossible.
The group of MI survivors was thus divided into two subgroups:
A — individuals with cholesterol synthesis in the interval of mean ± SD of the control group
B — individuals with cholesterol synthesis > mean ± SD.
Cholesterol synthesis in subgroup B was four times higher (1.30 pmol/10^6 cells/hr) compared to controls ($p < 0.001$) with a similar variation in both groups. No significant differences in any atherosclerosis risk factor were found when subgroups A and B were compared.
It is concluded that accelerated endogenous cholesterol synthesis may participate in premature atherosclerosis development in some individuals and contributes to the high sensitivity of responders to atherosclerosis risk factors.

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Lipoprotein profile and homocystein in patients with peripheral arterial occlusive disease

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Several studies have identified hyperhomocysteinemia as a possible risk factor for atherosclerosis. The purpose of a case-control study was to determine lipoprotein profile and homocysteine concentration in serum of 62 male patients with peripheral arterial occlusive disease and in 50 normolipidemic age-matched male controls. Cholesterol, triglycerides and phospholipids were measured within serum, HDL and subfractions HDL_2 and HDL_3, furthermore LDL cholesterol, Apo B, Apo A-I, lipoprotein particles Lp A-I, Lp A-I:A-II and Lp B:C-III. Homocystein was determined with the use of high pressure liquid chromatography. Patients with peripheral arterial occlusive disease showed significant higher mean concentrations of homocystein than control subjects (14.8 µmol/l vs 8.1 µmol/l). The prevalence of hyperhomocysteinemia (>16 µmol/l) in the patient groups was 34%. Lipoprotein profile showed only minor differences between the control and patient group. Triglyceride levels were elevated and Lp A-I particle concentrations were reduced. The results show that homocystein may be an important additional risk factor for peripheral arterial disease.

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Effect of smoking cessation on lipoprotein A-I and lipoprotein A-I:A-II levels

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Cigarette smoking is associated with low plasma high density lipoprotein cholesterol (HDL-C) and apolipoprotein (apo) A-I levels that may explain in part its deleterious effects on coronary heart disease (CHD). We investigated the influence of smoking cessation on the plasma apo AI-containing lipoproteins, taking into account the effect of environmental confounders such as body weight, nutritional intake and alcohol consumption. Since the latter variables influence Lp A-I levels on the one hand, and are affected by smoking cessation on the other hand, it was necessary to adjust for their potential confounding effects. We assessed in an ex-smoker group the influence of smoking cessation on apo A-I particle levels. The plasma lipid, apolipoprotein and lipoparticle concentrations of 58 subjects that completely stopped smoking (ex-smokers) were compared to those of 37 subjects that continued smoking (smokers) before and after a "Smoking Cessation Counselling Program". Nutritional intakes were recorded before and after the Program. Smokers and exsmokers were similar in gender distribution, age, body mass index, social status and nutrient intake. There were significantly greater increases in total cholesterol ($p < 0.04$), HDL-C ($p < 0.005$), HDL2-C ($p < 0.008$) and Lp A-I:A-II ($p < 0.04$) in exsmokers than in smokers. After smoking cessation, ex-smokers consumed more vegetable proteins ($p < 0.02$) and polysaccharides ($p < 0.04$) and had higher levels of HDL-C ($p < 0.0005$), apo A-I ($p < 0.002$), Lp A-I ($p < 0.007$) and Lp A-I:A-II ($p < 0.02$) than smokers. Adjustments on nutritional variables did not reveal any additional difference suggesting that smoking per se affects Lp A-I and Lp A-I:A-II levels. In conclusion, HDL particles including Lp A-I and Lp A-I:A-II are higher in exsmokers than smokers.

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Multiple modification of low density lipoprotein occurring in human plasma

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We have earlier found in the human blood a fraction of low density lipoprotein (LDL) which is characterized by a reduced content of sialic acid. Desialylated LDL also has a low neutral carbohydrate level, decreased content of major lipids, small size, high density, increased electronegative charge and altered tertiary apolipoprotein B structure. Unlike native LDL, this fraction of multiple-modified (desialylated) LDL induces the accumulation of lipids in smooth muscle cells of unaffected human aortic intima, i.e. exhibits atherogenic properties. In this study, we attempted to elucidate the mechanism of desialylation and other changes in the multiple-modified LDL by investigating the possibility of LDL modification by different cells and the blood plasma. A 24-hour incubation of lipoprotein with intact endotheliocytes, hepatocytes, macrophages and smooth muscle cells or cell homogenates at 37°C did not cause alterations either in the physical properties or chemical composition of native LDL. On the other hand, a significant fall in the lipoprotein sialic acid level was observed already after 1-hour incubation of native LDL with an autologous plasma-derived serum. While LDL sialic acid level continuously decreased, LDL became capable of inducing the accumulation of total cholesterol in the smooth muscle cells in 3 hour of incubation. Starting from the 6th hour of LDL incubation with serum, a monotonous decrease in the lipoprotein lipid content was observed as well as the related reduction of LDL size. Following 36 hours of incubation, an increase in the negative charge of lipoprotein particles was registered, too. Prolonged incubation (48 and 72 hours) of LDL with plasma-derived serum leads to the loss of atocopherol by the LDL as well as to their increased susceptibility to copper oxidation and accumulation of cholesterol covalently bound to apolipoprotein B, a marker of lipoperoxidation. Hence, desialylation of LDL particles represents one of the first or the primary act of modification which is, apparently, a sufficient prerequisite for the development of atherogenic properties. Subsequent modifications just enhance the atherogenic potential of LDL. The loss of sialic acid by LDL is occurred at neutral pH and was not inhibited by the lysosomal sialidase inhibitor 2,3-dehydro-2-deoxy-N-acetylneuraminic acid. The ^{3}H-labelled sialic acid removed from LDL was not found in free form, but in plasma fraction precipitated by trichloroacetic acid. This data suggest that enzyme(s) close to trans-sialidase play role in this process. Thus, this study demonstrated that the LDL modification processes imparting atherogenic properties to this lipoprotein can take place in the human blood plasma. Multiple modification of LDL is cascade of successive changes in the lipoprotein particle: desialylation, loss of lipids, reduction in the particle size, increase of its electronegative charge and peroxidation of lipids.

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Effect Of Native Low Density Lipoproteins On Nitric Oxide and Superoxide Production By Endothelial Cells

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Pathological modifications in biological systems induce acute and chronic alterations in the structural and functinal properties of the endothelium and modify the interaction between endothelial cells and the other molecules and cell types involved in the vascular tone homeostasis. Endogenous nitric oxide (NO) is the main factor involved in cardiovascular tone regulation, inhibition of smooth muscle proliferation, platelet aggregation and monocyte-macrophage activation. Low density lipoproteins (LDL) play a key role in endothelial cells injury and participate to the atherogenetic process also by affecting the endothelial-dependent vasodilation.

The aim of this study was to evaluate in situ the effect of increasing n-LDL concentration on endothelial NO and superoxide (O_2^-) production and clarify the precise extracellular concentration of LDL that leads to a modified or reduced activity of the L-arginine-NO system in the endothelial cell.

Endothelial human aorta cells were used for all the steps of the study and were grown to confluence in Dulbecco's modified medium (DMEM) containing 10% FBS and 1% antibiotic/antimycotic. LDL fraction was isolated by density gradient ultracentrifugation and dialysis against phosphate-buffer saline. NO levels were measured in situ by using an electrochemical method based on the oxidation of NO on a porphyrinic microsensor and measurement of the current generated from this process. O_2^- was detected by chemiluminescence. Endothelial cells were placed in the DMEM containing 5% lipoprotein deprived serum (LPDS) for 14–18 hours and later incubated for one hour with increasing LDL concentrations from 0 to 240 mg chol/dl) with and without L-arginine supplementation. In the next step, the endothelial cells have been tested in the same experimental conditions (increasing LDL concentrations) but pretreated with L-NAME (N^G-nitro-L-arginine-methyl-ester, 2×10^{-4} mol/L) for 30 minutes and superoxide desmutase (SOD, 100 U/mL) before NO and O_2^- were measured.

NO production already decreased sigmficantly at LDL concentration of 70-80 mg chol/dl, from 280 nM in LDL-free medium to 150 nM at the LDL concentration of 40 mg chol/dl, to 80 nM at the LDL concentration of 80 mg chol/dl; L-arginine pretreatment (10^{-6} Mol/L) did not totally block the inhibitory LDL effect on NO production but significantly increased NO levels at all the LDL concentrations. Furthermore, the SOD treatment did not modify the LDL effect on NO release.

The LDL treatment induced a sharp increase of O_2^- production in a dose-dependent fashion (from 10 nM at 0-30 mg chol/dL LDL to 90 nM at 80 mg chol/dL LDL). L-arginine supplementation did not increase the basal level Of O_2^- but produced a smaller O_2^- production at higher LDL concentrations. SOD supplementation did not cause a significant reduction of O_2^- production at every LDL concentration.

These results confirm the inhibitory role of LDL on NO production and suggest that the alteration of the L-arginine-NO system may be already present at normal LDL concentration. The amount of L arginine seems to be one of the main important steps for the optimal function of the NO system. We can hypothesize that hypercholesterolemia may damage the endothelial cell's membrane integrity and alter the transmembrane transport of L-arginine decreasing the substrate for the regular enzymatic activity of the constitutive nitric oxide synthase (cNOS).

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Macrophage-lysosomal enzyme relocalization and inactivation by oxidized LDL and protective effects of antioxidants

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The cytotoxicity of oxidized low density lipoprotein (oxLDL) to several types of artery wall cells might contribute to atherosclerosis by causing cell death, presumable by apoptosis. After its uptake, by receptor-mediated endocytosis, into macrophage lysosomes, oxLDL is poorly degraded, resulting in foam-cell formation. In order to identify the influence of oxLDL on lysosomal enzymatic activity and particular, on membrane stability, and its modulation by high density lipoprotein (HDL) and vitamin E J-774 cells and human monocyte-derived macrophages (HMDMs) were incubated for 24 to 48 h under normal culture conditions with either oxLDL alone or with oxLDL mixed with HDL or vitamin E. The lysosomal marker enzymes, cathepsin-L and N-acetyl-ß-glucosaminidase (NAßGase), were assayed after cell fractionation while cathepsin-D was demonstrated immunocytochemically. A test for lysosomal integrity, estimating the uptake of the lysosomotropic weak base acridine orange, was applied as well. We found that endocytosed oxLDL not only partially inactivated the lysosomal marker enzymes, but also destabilized the acidic vacuolar compartment and caused relocalization of lytic enzymes to the cytosol. It was further found that HDL and vitamin E, when mixed with oxLDL, significantly diminished the cytotoxicity of the latter compound. Cells exposed to ultraviolet-oxidized LDL enlarged and acquired a foam-cell morphology. Compared to normal control cells, the latter cells showed an enhanced amount of cytosolic cathepsin-D. The results indicate that lysosomal membranes and contents were damaged by oxLDL-exposure, resulting in partial relocalization of lysosomal enzymes. Such subcellular damage could be diminished by HDL and vitamin E.

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Serum antioxidant status in type 1 diabetic young patients

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In some studies, a decreased antioxidant activity has been shown in the serum of diabetic patients. The aim of the present study is to investigate parameters involved in cell defence against oxygen radicals (vitamin A and E), total antioxidant status (TAS Randox), autoantibodies against oxidized-LDL-lipoproteins (o-LAB-Elisa Biomedica) in relationship with diabetes duration, level of metabolic control (glycated hemoglobin-HbAlc), lipids abnormalities (total cholesterol, HDL-c, LDL-c, triglycerides) and subclinical complications (retinopathy, neuropathy, nephropathy). The study includes 110 young type 1 diabetic patients. The median age is 15 years (2 to 36 years) and the median duration of diabetes is 5 years (6 months to 30 years).

There is a positive correlation between age and vitamin A ($r = 0.5$; $p < 0.001$), TAS ($r = 0.23$; $p < 0.01$) and a negative correlation with o-LAB ($r = -0.28$; $p < 0.005$). The same relationships are found with diabetes duration. Serum vitamin E is independent of age or diabetes duration, is positively related to TAS ($r = 0.2$; $p < 0.05$) and negatively related to o-LAB ($r = -0.22$; $p < 0.05$). HbAlc levels are not related to o-LAB, vitamin A, vitamin E, or TAS. The patients with high serum lipids levels have the highest levels of serum vitamin A and E.When the patients are classified in function of the presence of subclinical complications, no significant differences are noted for o-LAB, vitamin E and TAS. Yet, the patients with nephropathy or neuropathy present higher values of vitamin A than the patients without complications. It is to be noted that vitamin A, vitamin E and TAS patients values are within the normal range but 33 patients present o-LAB values above the normal range.

Conclusion: Antioxidant status (TAS and vitamin A) of young type 1 diabetic patients change with age and diabetes duration, independently of the level of metabolic control. Autoantibodies against oxidized-LDL- lipoproteins decrease with age, as reported in healthy non diabetic patients.This decrease with age may be associated with the onset of atherosclerotic processes if we consider that o-LAB are an accompanying immunological expression of lipid peroxidation

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On the definition of premature corneal arcus

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Corneal arcus is both common and age dependent, but premature arcus is listed in many guidelines as an indication for lipid screening. What is premature? We have attempted to define this for a British population by assessing arcus expression in unselected patients consecutively attending a general practice, and in confirmed familial hypercholesterolaemia [FH] heterozygotes assessed at presentation before lipid therapy. Arcus was recorded by two experienced observers, on a previously established graded scale of 0–4, nil to full ring arcus. Progression with age for males, females and overall for the normal and FH groups was expressed in three ways: as the mean age of patients with each arcus grade, as the cumulative proportion of cases with a specified grade or any grade of arcus, and as the proportion of patients within age steps of 5 years with a specified grade or any grade of arcus to avoid any bias through age at recruitment on the cumulative distribution. For the 275 male, 351 female total 626 unselected patients age range 16–76 years, 50% had some degree of arcus of any grade by age 41–45 years, and by 31–35 years for the 59 male, 41 female total 100 FH of similar age profile. Heavy grade 4 arcus was present in 50% of the FH group by age 50 years and in the practice group by age 68 years, of whom 5% were then affected by age 50 years. Arcus progression was advanced by average 3 years in males for both the unselected and FH groups, this difference reducing with age, consistent with the increased proportion of cholesterol as LDL in males. These observations show that some degree of arcus is common in early middle age on assessment by experienced observers using good illumination, and confirm that premature heavy arcus has a variable relationship with but can alert to hypercholesterolaemia. This study also supports a broad definition of premature arcus for males and females combined as heavy full ring involvement by age 50 years.

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The discovery of leptin and the leptin receptor: Perspectives for the treatment of obesity and wasting disorders

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The ability to store large quantities of energy-dense fuel in the form of adipose tissue allows survival during prolonged periods of food deprivation. To maintain such fuel stores without undergoing continual alterations in body size and shape, a balance between energy intake and expenditure needs to be achieved. Leptin, the product of the *ob* gene, is secreted exclusively by white fat adipocytes, and appears to be the long sought after satiety factor released in the periphery to regulate long-term body weight. In this *lipostatic* theory of body weight maintenance, as body fat increases more leptin is secreted, thereby bringing the fat mass back to a certain normal set point. Once released into the circulation, leptin lowers body weight by decreasing appetite and altering metabolic processes. While the exact mechanism whereby leptin enters the brain needs to be elucidated, the hypothalamus appears to be the major target tissue for this hormone, since leptin receptors are present within the appetite control centers of this region, and since injection of leptin into the ventricular system of the brain reduces food intake and increases energy expenditure. Although the central mechanism of action has not yet been elucidated, leptin seems to alter energy expenditure by enhancing sympathetic outflow through modulation of synaptic transmission in the arcuate nucleus of the hypothalamus rather than through gene regulation. NPY, a neuropeptide known to increase food intake and body weight when injected in the brain, is coexpressed with the leptin receptor in the arcuate nucleus neurons and placed downstream in the biological response of leptin.

Leptin is present at high concentration in the serum of obese individuals, which suggests a central *resistance* to leptin, and brings into question the value of recombinant leptin for therapeutic applications. While the molecular mechanism for this resistance might perhaps be explained at the level of a defective transport to the brain or a defective leptin receptor signaling and/or translocation, a more plausable explanation is to be sought in defects of other components regulating energy expenditure, leading to a *saturation* of leptin. Clinically, leptin might however be effective for body weight maintenance after weight-loss through surgery (*virtual fat*). Antagonists of NPY or its hypothalamic receptor are currently under development for treatment of obesity. However, unlike for genetic animal models of obesity, there is no evidence for an increased central NPY level as a causative agent in human obesity. Recenly, genetic studies in mice have identified another component of the system that mediates weight control, the melanocortin-4 receptor, which when antagonized leads to obesity and lack of response to increased concentrations of leptin in the blood. Since this situation resembles the prominent leptin resitance observed in human obesity, this component will certainly receive much further attention for understanding and fighting this disease and all its complications. It has been demonstrated that inflammatory cytokines such as TNF, produces anorexia through the induction of leptin. This strongly advances the hypothesis that leptin may play a significant role in the anorexia and cachexia of inflammatory diseases and cancer. We have developed a human leptin antagonist which induces a progressive increase in body weight in normal mice. This antagonist could be of therapeutic use for wasting disorders.

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Epidemiology of risk factors for cardiovascular disease in diabetes and impaired glucose tolerance

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Diabetes, particularly non-insulin-dependent diabetes mellitus (NIDDM), is a strong risk factor for all manifestations of atherosclerotic vascular disease, coronary heart disease (CHD), cerebrovascular disease and peripheral vascular disease. Similarly, even milder abnormality in glucose metabolism, impaired glucose tolerance (IGT), increases the risk for atherothrombosis. Underlying mechanisms for the accelerated atherogenesis in NIDDM and IGT have remained poorly understood. NIDDM and IGT are known to be associated with several adverse cardiovascular risk factors, including hypertension, obesity, central obesity, hyperinsulinemia and serum lipid and lipoprotein abnormalities, characterized mainly by elevated serum total triglycerides and low high-density lipoprotein (HDL) cholesterol. Although much of the excess of CHD risk in NIDDM and IGT subjects can be accounted for by the high prevalence of these risk factors, a significant proportion of it remains unexplained. This strongly suggests that the excessive occurrence of CHD and other cardiovascular complications in NIDDM and IGT must be due to abnormal glucose tolerance itself or factors related to it. Only a limited number of studies on the risk for CHD in IGT subjects are available and therefore, the predictive value of different cardiovascular risk factors in IGT is difficult to evaluate. Recent studies on NIDDM patients demonstrate thai dyslipidemias, particularly low levels of HDL cholesterol and high levels of triglycerides are important risk factors for CHD. Also poor metabolic control significantly predicts CHD events. The risk factor profile for stroke is somewhat different. Hypertension is a strong risk factor for stroke similar to nondiabetic subjects. In addition, dyslipidemia (high cholesterol, low HDL cholesterol, high triglycerides) and particularly hyperglycemia are important predictors for stroke in NIDDM patients. The most important risk factor for peripheral vascular disease among NIDDM patients is poor metabolic control. In contrast to other manifestations of atherosclerotic vascular disease, dyslipidemia is not associated with the risk for peripheral vascular disease in NIDDM patients. In insulin-dependent diabetes mellitus the risk for atherosclerotic complications is predominantly linked with renal disease (micro- and macroalbuminuria).

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PPARγ and the molecular regulation of adipocyte differentiation

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Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear hormone receptor expressed predominantly in adipose tissue where it plays a central role in the control of adipocyte gene expression and differentiation. Since there are two additional PPAR isoforms, PPARα and PPARδ, and these are also expressed at some level in certain adipose depots, we have compared directly the adipogenic potential of all three receptors. Ectopically expressed PPARγ powerfully induces adipogenesis at a morphological and molecular level in response to a number of PPARγ activators. PPARα is less adipogenic but is able to induce significant differentiation in response to strong PPARα activators. This suggests that PPARα may play a role in differentiation of certain adipose depots in response to a different set of physiologic activators. Expression and activation of PPARδ did not stimulate adipogenesis. Of the three PPARs, only PPARγ can cooperate with C/EBPα in the promotion of adipogenesis. To begin to investigate the functional basis for the differential adipogenic activity of the PPAR isoforms, we have examined their ability to bind to several PPAR DNA response sequences. Compared to PPARα and PPARδ, PPARγ shows preferential binding to two well characterized regulatory sequences derived from a fat-specific gene. We have also begun construction of chimeric receptors to localize the domain of PPARγ which is responsible for its adipogenic activity. Using PPARδ as a backbone, we have localized an adipocyte differentiation domain in the amino-terminal half of PPARγ which will confer adipogenic activity to PPARδ. This region includes a MAP kinase phosphorylation site through which many growth factors negatively regulate PPARγ, thereby repressing adipocyte differentiation.

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Plasma levels of lipid and cholesterol oxidation products and cytokines in diabetes mellitus and cigarette smoking.

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The relation between an increased LDL cholesterol concentration and premature coronary atherosclerosis is clearly established. However, at a given cholesterol concentration there is a large variation in the expression of the disease. The reason for this phenomenon is largely unknown, but not only the LDL cholesterol level itself, but also postsecretory modifications of LDL and changes in immune mechanisms may be critical in the process of atherosclerosis. To evaluate the role of both oxidation and inflammation in atherosclerosis, we compared LDL oxidizability, *in vivo* lipid and cholesterol oxidation and basal and lipopoly-saccharide (LPS-) stimulated production of various cytokines in normolipidemic patients with diabetes mellitus (DM; n = 11), smokers of cigarettes (n = 12) and controls (n = 14). In addition, the effects of vitamin E (600 IU/day for 4 weeks) on these parameters were evaluated. The oxidizability of LDL can be measured by several methods, of which the most frequently used are estimation of the amount of thiobarbituric acid reactive substances (TBARS) formed by the lipid peroxidation process; and estimation of the amount of conjugated dienes formed in vitro during incubation with copper ions monitored by measurement of the change of absorbance at 234 nm.

LDL oxidation characteristics before and after vitamin E were identical in the 3 groups. Plasma thiobarbituric acid reactive substances were higher in DM and smokers vs. controls (0.77 ± 0.22, 0.74 ± 0.14 vs. 0.62 ± 0.10 μmol malodialdehyde equivalents/l, respectively; P vs. controls < 0.05) and normalized after vitamin E suppletion. Total plasma oxysterols were higher in smokers vs. controls (354 ± 104 vs. 265 ± 66 nmol/l, $P < 0.05$) and unaffected by vitamin E.

The basal and LPS-stimulated levels of interleukin-1ß and tumour necrosis factor α (TNFα) and the basal level of interleukin-1-receptor antagonist (IL-IRA) were identical for the 3 groups. LPS-stimulated IL1-RA was higher in DM vs. controls 10.7 ± 2.0 vs. 8.1 ± 1.7 pmol/l, $p < 0.05$). After vitamin E, TNFα dropped in controls and smokers, and IL1-RA in smokers only.

The results suggest that ther is an increased *in vivo* oxidative stress and inflammation in DM and smokers, which is partly overcome by vitamin E.

Whether antioxidants may be of use in the prevention of atherosclerosis remains to be determined.

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Nuclear receptors as regulators of lipid metabolism

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The peroxisome proliferator activated receptors (PPAR), α, β, and γ, with each a specific tissue distribution, compose a subfamily of the nuclear hormone receptor gene family. Fibrates and leukotriene B4 are ligands for PPARα, whereas prostaglandin J12 derivatives and thiazolidinedione antidiabetic agents are ligands of PPARγ. Activated PPARs heterodimerize with RXR and alter the transcription of target genes after binding to specific response elements or PPREs, consisting of a direct repeat spaced by 1 nucleotide. PPARγ triggers adipocyte differentiation by inducing the expression of several genes critical for lipid storage and adipogenesis. PPARα is involved in stimulating β-oxidation of fatty acids, a process which lies in rodents at the basis of the phenomenon of peroxisome proliferation. Because PPARα stimulates the catabolism of pro-inflamatory fatty acid derivatives, such as leukotriene B4, it has anti-inflammatory properties. PPARα is also involved in the control of HDL cholesterol levels, an effect mediated by transcriptional regulation of the major HDL apolipoproteins, apo A-I and apo A-II. The hypotriglyceridemic action of PPARα and γ activators is mainly caused by an increased lipolysis and clearance of triglyceride (TG)-rich particles, due to changes in lipoprotein lipase and apo C-III levels. Whereas thiazolidinediones predominantly affect adipocyte LPL production through activation of PPARγ, fibrates and fatty acids exert their effects mainly in the liver via a PPARα-mediated reduction in apo C-III production. PPARα activators also lower circulating TG levels by the following additional mechanisms confined to the liver: 1) a stimulation of cellular fatty acid uptake and their conversion to acyl-CoA derivatives by the induction of FATP and ACS activity, 2) an induction of fatty acid β-oxidation pathways, 3) a reduction in fatty acid and TG synthesis and finally 4) a decrease in VLDL production. Hence, both enhanced catabolism and reduced secretion of TG-rich particles are mechanisms that contribute to the hypolipidaemic effect of fibrates, whereas thiazolidinediones only enhance catabolism of VLDL particles. For PPARβ sofar no function has been identified. The different PPARs are key messengers responsible for the translation of nutritional, pharmacological and metabolic stimuli into changes in the expression of genes, more specifically those genes involved in lipid metabolism.

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Candidate Genes in Non-Insulin-Dependent Diabetes Mellitus

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NIDDM is a heterogeneous disorder characterized by impaired pancreatic, β-cell function, peripheral insulin resistance, and overproduction of glucose by the liver. Genetic and familial factors contribute significantly to the susceptibility to this disease. It is very likely that multiple genetic factors contribute to the overall risk. We have attempted to dissect the genetic factors predisposing to NIDDM susceptibility by focusing on specific candidate genes whose products are known to play a role in insulin secretion and in resistance to the action of insulin. We have used Japanese-American and Finnish patient and control populations to screen for sequence variants in these candidate genes and examined if such variants are associated with any aspects of the disease.

We found that a G —> A substitution at position -30 of the β-cell glucokinase gene promoter was over-represented in individuals with impaired glucose tolerance. Furthermore, we observed that this substitution is associated with reduction in β-cell function (diminished relative insulin response) in middle-aged Japanese-American men, and may in part explain the high risk of abnormal glucose tolerance in this population. This promoter variant was also observed to be relatively common among populations of Northern European extraction as well as among African-Americans.

We also observed an association between the Trp 64 Arg substitution in the β_3-adrenergic receptor with abnormal glucose tolerance but not wlth fasting insulin levels and body fat distribution among JapaneseAmerican men.

The association of markers at other candidate gene loci with NIDDM will be discussed.

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Human obesity: from lipid abnormalities to lipid oxidation

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The obese state has been recognized to accentuate the known risk factors for atherosclerotic disease as dyslipidemia, hypertension, glucose intolerance and insulin resistance.

Among other risk factors, obesity is characterized by a series of lipid disturbances, such as hypercholesterolemai, high fasting (and postprandial) triglyceride levels, low HDL cholesterol, high apolipoprotein B, high small dense lipoprotein particles and alterations of serum and tissue LPL-activity.

Although obesity is associated with such cluster of lipid abnormalities, these factors do not explain the complete process of atherogenesis in the obese subject. Other risk factors belonging to the polymetabolic syndrome-cluster, insluin resistance, hypertension, fibrinogen, add substantial but not full explanation to the atherothrombotic process. Over the last decade, a series of excellent studies have provided the background for a more indepth mechanism of atherosclerosis; the role of lipid peroxidation in particular has been one of the focuses of this current research.

There exists a lot of evidence suggesting a major role for oxidation of LDL and VLDL particles in the pathogenesis of atherosclerosis.

Although obesity is characterized by dyslipidema, less is known about the oxidation capacity of lipoproteins in obese subjects. The oxidizability in vitro, in premenopausal women and compared to age-matched controls, will be discussed.

The oxidizability of the non-HDL fraction is evaluated by measuring the fluorescence and thiobarbituric acid reactive substances (TBARS; MDA nM/mg non-HDL) at different time intervals of incubation.

TBARS formation increased linearly with the increase of lipids both in non-obese and obese subjects. TBARS, measured every 30′, increased in non-obese controls up to a max. of 59.6 at 180′ in contrast to a max. of 77.1 at 180′ ($p < 0.001$) in obese subjects.

Also the lag-time (period from zero to the start of the particle oxidation process) was significantly lower (92.5 vs. 123.4; $p < 0.001$) in obese subjects, when compared to lean controls.

BMI correlates significantly with TBARS formation and its log transformed values (max $p < 0.001$). The lag-time was negatively related to body weight and BMI and the waist-to-hip ratio. A significant relationship exists between TBARS formation (up to $r = 0.59$) and triglyceride levels and a negative relationship exists with HDL-cholesterol levels.

In vitro oxidizability of non-HDL lipoproteins is significantly increased in obese, non diabetic subjects and related to increase body weight reduction and anti-oxidants ingestion.

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Effect of TaqIb and I405V polymorphisms in the cholesteryl ester transfer protein (CETP) on plasma level of HDL and apoAI and postprandial triglyceride level.

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The effect of two common polymorphisms of the cholesteryl ester transfer protein (CETP), the TaqIB in intron 1 and isoleucine 405 to valine (I405V) was examined in a sample of 794 men age 22–25 from 12 countries in Europe that participated in the European Atherosclerosis Research Study II (EMS II). PCR based methods were used for genotyping of both the polymorphisms and the frequency of the rare Taq lB allele (+ or A) and the rare V405 were 0.44 and 0.27 respectively and were not different in different regions of Europe. The sample was in Hardy Weinberg equilibrium for both polymorphisms. There was a statistically significant effect of the TaqlB polymorphism on both plasma HDL cholesterol (HDLC) and apoAI level ($p < 0.001$), with those homozygous for the rare A allele having the highest level. Those individuals homozygous for the rare V405 allele had the highest HDLC and apoAI levels, though this effect only reached statistical significance for HDLC ($p < 0.03$). TaqlB explained 3.7% and I405V 0.9% of the variance in HDLC. Together the two polymorphisms explained 4.1% of the variance in HDLC and are thus roughly additive. There was no significant effect of the polymorphisms on other baseline lipid parameters, and no statistically significant interaction with smoking or alcohol consumption in these young men. An oral fatload test was carried out on all the subjects of the study. When the effect ofthe polymorphisms was examined for the postprandial triglyceride (TG) level, those individuals with the common genotypes and the lowest fasting HDLC had the highest TG levels at all times postprandially, though this effect was not statistically significant. Hov ever, when those individuals with the TaqlB A allele, who had the highest HDLC ($n = 132$) were examined according to the I405V genotype, those homozygous for both the TaqlB A and the V405 alleles ($n = 28$) had significantly lower TG, as well as area under curve and peak height for postprandial TG after oral fat load than those carrying the c-mmon I405 allele in that group ($n = 124$) ($p < 0.03$ for all parameters). There was a statistically significant interaction of the TaqlB and I405V polymorphisms for the postprandial TG level ($p < 0.04$). These results suggest that the TaqlB and I405V polymorphisms represent two independent functional variations in the CETP gene that may modify the postprandial TG level after oral intake of fat.

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Gene with gene interaction causes changes in plasma lipoprotein concentrations

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Prevalent missense mutations in the genes encoding apolipoprotein (apo) E (Arg158Cys), lipoprotein lipase (LPL) (Ser447End), and cholesterol ester transfer protein (CETP) (Ile405Val) have each been shown to be associated with significant increases of serum HDL-cholesterol. As the associated plasma lipid changes are small (5–10%) these genetic variants will only be useful in individual risk prediction if the observed phenotypic effects of the mutations are additive. We have tested the interaction of the CETP and the apo E mutation and found that male individuals with wild-type sequence at both mutant positions had the lowest HDLcholesterol concentrations (46.7 mg/dl). Heterozygosity for either the CETP mutation or the apo E mutation were intermediate (48.9 mg/dl for both mutations). Individuals heterozygote at both positions had the highest HDL-cholesterol levels (52.4 mg/dl). Variance analysis showed significant differences ($p = 0.033$). A significant positive interaction was also seen upon variance analysis of the interaction between the LPL and the apo E mutations ($p < 0.001$) and between the CETP and the LPL ($p < 0.001$) mutations. A variance analysis which included all three mutations also showed significant positive main-effects ($p = 0.001$). In order to simplify the analysis we formed groups of individuals who had mutations in none, one, two or all three of the above genes. The HDLcholesterol concentrations in these groups were 45.9 ($n = 231$), 48.8 ($n = 258$), 52.3 ($n = 77$), and 52.7 ($n = 7$), respectively. All differences between the first three groups were significant ($p < 0.05$; t-tests). Our data demonstrate that the presence of several mutations with small effects in one individual can result in major phenotypic changes. They provide a first step towards the understanding of complex disorders.

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Effect of adenovirus mediated overexpression of human phospholipid transfer protein (PLTP) on the plasma lipoprotein profile in different strains of transgenic mice

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Numerous studies have shown a strong inverse relationship between high-density lipoprotein (HDL) levels and atherosclerosis. This has been attributed to the role of HDL in the reverse cholesterol transport. PLTP has the ability to transfer phospholipids between lipoprotein classes and, in addition, it mediates conversion of HDL and generates preβ-HDL, the primary acceptor of cell membrane cholesterol. To reveal the physiological function of PLTP, we used adenovirus-mediated gene transfer to study the effect of overexpression of PLTP on lipoprotein metabolism in three different strains of mice: wild type (C57/BL), human apoA-I transgenic (HuA-I Tg) and human apoE3Leiden-E-knock-out-LDL-receptor knock-out heterozygous mice (apoE-Leiden Tg). The mice received 6×10^8 pfu of adenovirus containing the human PLTP cDNA driven by a CMV promotor and were analyzed on day five.

PLTP activity increased 10–20 fold in the three strains. As a result the C57/BL mice showed an almost complete lack of plasma cholesterol (CHOL) and triglycerides (TG). HuA-I Tg had a dramatic decrease in CHOL and TG (19 % and 50% respectively). ApoE-Leiden had a large increase in TG and CHOL (88% and 55% respectively) confined to the fractions corresponding to VLDL. Immunoblotting of the fractions showed an absence of apoA-I, apoB and apoE were detected in the VLDL and LDL fractions.

The results demonstrate that the overproduction of PLTP by adenovirus infection exerts a dramatic effect on the quantitative distribution of the different lipoprotein fractions. This may be due to a displacement of apoA-I from the HDL leading to a more rapid clearance of apoA-I. The mechanisms behind these changes remain to be investigated.

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Plasma phospholipid transfer protein interacts with the aminoterminal portion of apoA-I

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Pig plasma phospholipid transfer protein (PLTP) and human plasma PLTP have similar molecular weights, phospholipid transfer activity and HDL conversion activity. The sequences of the two proteins share 93% identity.

The interaction of PLTP with HDL has not been characterized in detail. We have reported that apoA-I/apoA-II molar ratio in the HDL particle is important for PLTP function.

The aim of the study was to examine whether PLTP binds to apoA-I and to characterize the binding region.

To study the PLTP/apoA-I interaction we used the ELISA-technique. The plates were coated with 5 μg/ml of lipid-free human apoA-I, and incubated with serial dilutions of PLTP. The interaction was monitored with peroxidase labelled monoclonal anti-PLTP antibody, G11. PLTP bound to apoA-I in a concentration dependent manner. Free apoA-I competed for this binding.

PLTP did not bind to human serum albumin (HSA), nor was HSA able to interfere with the PLTP/apoA-I interaction. To analyze to which portion of apoA-I PLTP binds, ELISA plates were coated with recombinant full-length apoA-I (residues −6–243) or three truncated forms, terminating at amino acid residues 222,150 and 135. The binding of PLTP to the full-length apoA-I and to three truncated forms of it was similar suggesting that the binding of PLTP is to the N-terminal portion of apoA-I. Truncated apoA-Is also competed for this binding. To further substantiate this result, we used polyclonal (R33) and several monoclonal anti-apoA-I antibodies (kind gifts from Drs. N.Fidge and Y.Marcel) to map the binding region of PLTP in apoA-I. The antibodies which inhibited the interaction between PLTP and apoA-I were directed towards apoA-I epitopes localized between amino acids 27–141 (A-1-1, 2G11, 3G10, 3D4 and 5F6). The polyclonal antibody, R33 and the monoclonal antibody A-1-1 (amino acids 27–47) were the most effective and reduced PLTP binding by 70% (at Ab concentration of 0.1 μg/ml). These results suggest that PLTP interacts with the aminoterminal portion of apoA-I.

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Temperature dependence of the phospholipid transfer protein- mediated transfer of phospholipids from liposomes to high density lipoprotein

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Plasma lipoproteins are continuously remodeled through the actions of enzymes and of specialized lipid transfer proteins. The transfers of cholesterol esters and triglycerides are mediated by the cholesterol ester transfer protein (CETP), while that of phospholipids is facilitated by both CETP and the phospholipid transfer protein (PLTP). In contrast to CETP, PLTP has been reported to be a thermolabile protein. The present work was undertaken to study the temperature dependence of PLTP-mediated phospholipid transfer (PLT). PLT was measured as the unidirectional transfer of ^{14}C-labelled dipalmitoylphosphatidylcholine from sonicated phospholipid liposomes to high density lipoprotein. In this assay, only PLTP (but not CETP) is able to facilitate PLT. We therefore used a d > l.21g/ml plasma fraction as the source of PLTP. In each assay, the PLTP-facilitated PLT was calculated as the difference between the PLTs obtained respectively in the presence or in the absence of PLTP. Kinetics of PLT (up to l h30) were measured at different temperatures ranging from 4 to 65 C. From the initial velocities of the reaction, we calculated the respective first-order rate constants for spontaneous and PLTP-facilitated PLT. Both rate constants increased progressively with temperature, giving linear Arrhenius plots, which indicated a simple thermodynamic effect of temperature. When PLTP-facilitated PLT was measured after incubations longer than 45 min, the effect of temperature was complex. PLT values increased from 4 to 37 C, then decreased until 50 C and finally increased again untill 65 C. These data suggested that long term PLT values resulted from two opposite effects of temperature : increase of PLT rate constants and heat inactivation of PLTP. Heat inactivation of PLTP was measured as follows. PLTP was pre-incubated for l h at various temperatures. PLT was then measured after 10 min incubations at 37 C. No denaturation of PLTP was observed until 37 C. Preincubations of PLTP above 37 C led to a biphasic heat inactivation process. Between 37 and 45 C, PLTP lost 30% of activity. No further inactivation was observed untill 50 C. Then the second phase of heat denaturation took place, leading to a progressive inactivation of PLTP. No residual activity was found above 60 C. These data suggest that heat inactivation may differentially affect two distinct parts of the PLTP molecule, which are both involved in the phospholipid transfer process.

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Lipoprotein lipase −93T/G transition is associated with lower plasma triglyceride levels and increased promoter activity *in vitro*.

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We have used SSCP to screen for mutations in the LPL promoter, from the translation start site to nt −701. A single common mutation, −93T/G transition, was identified. The frequency of this mutation is 0.03 (n = 998) in healthy middle-aged men and 0.08 (n = 230) in FCHL and MI patient groups. There is a very strong association between −93T/G and D9N, 83% of −93G mutations are found in conjunction with the N9 mutation; thus all N9 mutations are on a −93G allele, but not all −93G are on an N9 allele. We examined the effect of the −93G on plasma Tg levels in the healthy men; these were consistent over a four year study period. Carriers of −93G/D9 have significantly lower Tg levels than wild-type −93T/D9 individuals. As identified before, N9 carriers, who have now been identified as always carrying the −93G, have significantly higher Tg

−93T-G/D9N	TT/DD	TG/DN	GG/DD
N	965	27	6
Tg (SEM) (mmol/l)	1.79 (0.85)	2.09 (0.33)	1.19 (0.12)

Overall these results reached statistical significance ($p < 0.04$)

In vitro expression of −93 variants, using the luciferase reporter gene system in a smooth muscle cell line A10, showed the −93G construct to have 24% higher promoter activity than the −93Tconstruct. This increased promoter activity could account for the lower Tg levels in −93G/D9 carriers. Thus −93G might in fact mask the true lipid raising effect of N9 *in vivo* in −93G/N9 carriers. We have also studied these mutations in patients with NIDDM. The impact on Tg levels is the same and preliminary results suggest that −93G is associated with lower BMI and may reduce the hypertriglyceridaemia in diabetic patients.

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Role of the ARG^{123}-TYR^{166} paired helix of APO A-I in lecithin:cholesterol acyltransferase activation

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The Arg^{123}—Tyr^{166} central and the Ala^{190}—Gln^{243} carboxyl-terminal pair of helices of apo A-I were substituted with the pair of helices of apo A-II, resulting in the apo AI[Δ(R^{123}—Y^{166}), ∇A-II(S^{12}—A^{75})] and the apo A-I[Δ(A^{190}—Q^{243}), ∇A-II(S^{12}—Q^{77})] chimera, respectively. The structure of these chimeras in aqueous solution and in reconstituted HDL (rHDL) and the lecithin:cholesterol acyltransferase (LCAT) activation properties of the rHDL were studied. Recombinant human apo A-I and chimeras were expressed in *Escherichia coli* and purified from the periplasmic space. Binding of the apolipoproteins with palmitoyloleylphosphatidylcholine was associated with a similar shift of Trp fluorescence maxima from 337 to 332 nm, from 339 to 334 nm and from 337 to 333 nm, respectively. All rHDL had a Stokes radius of 4.8 nm and contained 2 apolipoprotein molecules per particle. Circular dichroism measurements revealed 8 α-helices per apo A-I and per chimera molecule. Recombinant LCAT was obtained by cloning of the LCAT cDNA into the pcDNA expression vector followed by transfection into 293 cells and selection of transformed cells in the presence of geneticin. Colonies were screened for LCAT expression by spectrophotometrical assessment of the hydrolysis of the fatty acid esters of p-nitrophenol. The catalytic efficiencies for LCAT activation were 1.4 ± 0.27 nmol CE/h.μM (mean ± SD; n = 3) for apo A-I, 0.053 ± 0.008 nmol CE/h.μM ($p < 0.001$ vs apo A-I) for the central domain chimera and 1.3 ± 0.30 nmol CE/h.μM (p = NS vs apo A-I) for the carboxyl-terminal chimera. The lower LCAT activity of the central domain chimera was due to a 25-fold reduced V_{max} with unaltered K_m. In conclusion, although substitution of the Arg^{123}-Tyr^{166} central or of the Ala^{190}-Gln^{243} carboxyl-terminal pair of helices of apo A-I with the pair of helices of apo A-II yields chimeras with similar secondary structure as native apo A-I, exchange of the central but not of the carboxyl-terminal domain of apo A-I reduces the rate of LCAT activity that is independent of the binding to rHDL.

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The incidence of C-T substitution at −480 of the hepatic lipase (HL) promoter in patients with mixed hyperlipidemia

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Jansen et al(1) have reported a polymorphism, C-T at position −480, in the promoter of the HL gene. The incidence of the T allele in a group of 700 patients with coronary artery disease (CAD) was 0.22. The T allele was associated with lower HL activity and TT homozygotes had significantly higher HDL levels. The levels of other lipids were not significantly different. We have determined the frequency of this polymorphism in a group of 113(79 male, 34 female) patients attending the lipid clinic at the University Hospital in Hamburg. 66 patients were CC, 40 CT and seven TT, a frequency for the T allele of 0.24. There were no significant differences in TC (282 vs 301) or TG (547 vs 607) between CC and CT patients. Eight of the CC and four of the CT patients had CAD. These frequencies are similar to those reported by Jansen et al. In contrast, we did not detect significantly higher HDL levels in TT patients. The HDL levels of the seven TT patients were 20, 21, 20, 40, 27(males) and ? and 63 (females), compared to mean levels for CC patients of 29.2(males) 34.5(females) and 31.5(males) and 48.4(females) for CT patients. The apoE genotypes were determined. Three of the TT patients were apoE 3/3, one 3/4, one 2/2 and one was heterozygous for the rare mutation, R136C. We also determined the incidence ofthe lipoprotein lipase (LpL) polymorphisms, −93 T-G, D9N and N291S. 11 patients were heterozygous for the −93 T-G of which 9 were also heteozygous for D9N. There were 15 hetero- and one homozygote for N291S. Of the TT patients, one was heterozygous for −93 T-G and D9N and was apoE 3/4. One was heterozygous for N291S and also heterozygous for the rare LpL variant, V69L. Thus of seven TT patients, three also had rare variants in either LpL, apoE or both and a fourth was apoE 2/2 with Type III hyperlipoproteinemia. This additional variation may account for the differences between our results and those of Jansen et al. These results illustrate the difficulty in showing the effect of an individual polymorphism in a candidate gene on the lipid phenotype since lipid levels are determined by the complex interaction of a number of genetic and environmental factors. The effect of variation in one gene may thus be masked by additional variants in other genes particularly in patients with hyperlipidemia.

1. H.Jansen etal. Abstract 66th Congress of the EAS, Florence, 1996

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Lipoprotein lipase is required for the binding of very low density lipoproteins to heparan sulphate proteoglycans

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Beside the involvement of heparan sulphate proteoglycans (HSPG) in the binding of lipoprotein lipase (LPL) and thus in the lipolysis of very low density lipoproteins (VLDL), HSPG are also thought to play a role in the binding and clearance of VLDL. In literature two possible mechanisms of interaction have been described: (i) apolipoprotein E (apoE)-mediated binding and (ii) LPL-bridging. In order to discriminate between these mechanisms, binding experiments were performed using microtiter plates coated with HSPG or HSPG/LPL complexes.

VLDL (d < 1 .006 g/ml) was isolated from a human apoE3 homozygote, apoE-deficient mice (*apoe*−/−), apoE2(Arg158➔Cys) and apoE3-Leiden mice both without endogenous apoE (APOE*2•*apoe*−/− and APOE*3-Leiden•*apoe*−/−, respectively) and control mice (*apoe*+/+). The binding of all ^{125}I-labelled VLDL samples to plates coated with HSPG only was barely measurable, whereas VLDL binding to plates containing HSPG/LPL complexes was increased up to 70-fold. Under the latter conditions, both human E3E3 VLDL and mice *apoe*+/+ VLDL displayed the highest affinity as calculated by Scatchard analysis (K_a = 5.88 ml/μg and 7.69 ml/μg, respectively). *Apoe*−/− VLDL showed the lowest binding affinity (K_a = 0.88 ml/μg) to HSPG/LPL complexes, but was still able to bind despite the absence of apoE.

The presence of an excess of apoE as found on APOE*2•*apoe*−/− VLDL particles (15.57 apoE molecules per particle) showed no higher affinity to HSPG as compared to human E3E3 VLDL (containing only 0.45 apoE molecules per particle). Our results indicate that HSPG binding of VLDL is hardly mediated by apoE, but requires the bridging of LPL.

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Physiological doses of chemically different antioxidants decrease the plasma level of oxidized cholesterols in hyperlipidaemic rabbits

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There is strong evidence that the oxidation of plasma lipoproteins plays a key role in atherogenesis. Supplementation with antioxidants has been shown to decrease lipoprotein oxidation in vitro and is therefore expected to delay the development of atherosclerosis. However, it is unknown whether supplementation with physiological doses of antioxidants is sufficient to decrease lipoprotein oxidation and retard atherogenesis in vivo. We studied the influence of antioxidants on lipoprotein oxidation in vivo in hyperlipidaemia, which is a known risk factor for atherosclerosis. Watanabe heritable hyperlipidaemic rabbits were used as an animal model of hyperlipidaemia. The rabbits were supplemented with physiological amounts of chemically different antioxidants (4.3 mg of α-tocopherol, 4.3 mg of ubiquinone-10, 75 mg of carvedilol or 15 mg of probucol/kg body weight daily) for 3 months. Plasma level of oxidized cholesterols (7-, 20- and 25-hydroxycholesterols, 5α- and 5β-epoxycholesterols and 7-ketocholesterol) was measured as an indicator of lipoprotein oxidation in vivo. We found that the supplementation with ubiquinone-10 significantly decreased the plasma level of 7- and 25-hydroxycholesterols and 5β-epoxycholesterol. Supplementation with carvedilol significantly decreased plasma level of total oxycholesterols, total hydroxycholesterols, 7- and 25-hydroxycholesterols and 5β-epoxycholesterol. Supplementation with α-tocopherol and probucol were ineffective in decreasing oxycholesterols in rabbit plasma. These data indicate that supplementation with physiological amounts of the chemically unrelated antioxidants ubiuqinone-10 and carvedilol is able to decrease the in vivo lipoprotein oxidation in hyperlipidaemic animals. This suggests that antioxidant supplementation may represent an efficient therapeutic tool to retard atherogenesis in vivo.

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Familial Lecithin: Cholesterol Acyltransferase Deficiency: Molecular analysis of a compound heterozygote: LCAT (Arg^{147}→Trp) and LCAT (Tyr^{171}→ Stop)

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LCAT is responsible for the formation of the majority of plasma cholesteryl esters. Familial LCAT deficiency is associated with corneal opacity, anemia and proteinurea and typically results in renal failure in the 4–5th decade; this syndrome is equally characterized by the quasi-absence of plasma LCAT activity with variable enzyme mass and very low levels of plasma cholesteryl esters. In this study, we report detailed analyses of plasma lipids and lipoprotein profile in two sisters (CM and ML) presenting classical homozygous LCAT-Deficiency; the younger sibling (CM) had proteinurea from an early age whereas the older sister (ML) has never exhibited renal dysfunction. We investigated the molecular defect in the 45 year-old woman (proband CM) exhibiting all clinical and biochemical features of Familial LCAT Deficiency: a plasma cholesterol level of 105 mg/dl, of which 95% was unesterified, an HDL-cholesterol of 6.5 mg/dl and an Apo AI level of 52 mg/dl. The proband (CM) displayed a plasma cholesterol esterification rate which corresponded to 2% of normal LCAT activity; plasma LCAT protein concentration was 0.56 µg/ml and equivalent to approximately 10% of normal LCAT mass. Analysis by Single Strand Conformation Polymorphism (SSCP) of the PCR products corresponding to exons 4 and 5 of the LCAT gene revealed a visible band shift. Sequence analyses of exons 4 + 5 revealed two separate single point mutations: a C→T transition replacing Arg^{147} by Trp and a T→G transition convertingTyr^{171} to a stop codon. The presence of these two point mutations was confirmed by restriction enzyme analyses: the C→T transition abolished a MwoI site whereas the T→G transition created an AvrII site. The Arg^{147} mutation was associated with a non-secreted protein. The Tyr^{171} mutation resulted in formation of a truncated protein lacking the catalytic site. In summary, we have identified an LCAT deficient patient corresponding to a compound heterozygote for the Arg^{147}→Trp mutation and a new molecular defect involving a Tyr^{171}→Stop mutation in the LCAT gene.

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Managing Global Risk: A New Therapeutic Objective

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Data from the Münster Heart Study (PROCAM) has allowed calculation of a risk algorithm taking into account the independent variables age, systolic blood pressure (SBP), LDL cholesterol, HDL cholesterol, triglycerides (TG), cigarette smoking (y/n), family history of diabetes mellitus (y/n), and family history of MI (y/n). The relative importance of continuous risk variables in Münster was: LDL > total cholesterol > HDL > TG > fasting blood glucose > SBP > body mass index. Additional independent risk factors yet to be incorpoarated in the algorithm are fibrinogen, clotting factor VIIc and Lp(a). In the PROCAM, Framingham and MRFIT studies, a log-linear relationship existed between LDL and CHD risk. Thus when the **relative** risk of CHD is plotted against LDL cholesterol, a straight line relationship is obtained. However, the risk associated with a given LDL cholesterol depends of the presence of other CHD risk factors. Thus, a person with an LDL cholesterol of 250 mg/dl without other risk factors might have the same CHD risk as a person with an LDL cholesterol of only 150 mg/dl but with additional factors. In *both cases*, a prolonged lowering of cholesterol of about 40 mg/dl is associated with about a halving of CHD risk. Because of the overriding importance of **global risk**, treatment must include modification of as many adverse variables as possible by e.g. by losing excess weight, giving up smoking, taking exercise etc. Since these risk factors interact in a multiplicative fashion, small changes in a number of risk variables may produce a disproportionately large overall benefit.

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Small, Dense LDL: Atherogenic impact and new therapeutic approaches

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The Framingham Heart Study has clearly established that elevated plasma levels of low-density lipoproteins (LDL) significantly increase the risk of premature coronary artery disease (CAD). Furthermore, recent primary and secondary intervention trials involving use of hypolipidemic drugs have demonstrated the markedly beneficial effects of LDL lowering on reduction in clinical events. Only recently has attention been drawn to the qualitative features of plasma LDL particles: nonetheless, abundant evidence has been provided to substantiate the abnormal quality of LDL particles in CAD patients, which are characterised by a predominance of small, dense LDL.

Plasma LDL are cholesterol-rich particles containing apolipoprotein B100 which were originally considered to consist of a homogenous population. Multiple subpopulations of LDL particles, which differ in their physicochemical properties, metabolism and biological functions, are however present within the LDL density range of 1.019 to 1.063 g/ml. These different LDL subpopulations may be grouped into three major subclasses: light, large LDL (d 1.02–1.03 g/ml), intermediate LDL (d 1.03–1.04 g/ml) and small, dense LDL (d 1.04–1.06 g/ml).

Hypercholesterolemia, combined hyperlipidemia and hypertriglyceridemia are dyslipidemias associated with premature vascular disease and alherogenic lipoprotein phenotypes. Indeed each of these dyslipidemias features either an elevated plasma concentration, or an increased proportion, of dense LDL. These particles are highly atherogenic as a result of their low binding affinity for the cellular LDL receptor, their prolonged plasma half-life and their diminished resistance to oxidative stress. Biological modification of dense LDL is potentiated as a result of retention in the arterial intima upon binding to extracellular matrix components, leading to uptake by macrophages with subsequent foam cell formation.

The biological mechanisms which underlie the formation of dense LDL involve pathways of lipoprotein synthesis, assembly and secretion as well as tissue degradation via specific receptors; lipolytic enzymes and lipid transfer proteins are equally implicated as major components in the intravascular remodelling of lipoprotein particles, among which the cholesteryl ester transfer protein (CETP) plays a key role. The pharmacologic modulation of the plasma concentrations and physicochemical properties of dense LDL is of special interest, and represents a new therapeutic approach in the treatment of the atherogenic dyslipidemias. Recent clinical studies of the impact of fibrate derivatives on the atherogenic LDL profile in Combined Hyperlipidemia have revealed a significant degree of normalisation, with reduction of up to 50% in dense LDL levels. Our findings suggest that a key feature of such action involves reduction of elevated CETP-mediated cholesteryl ester transfer from HDL to VLDL particles. The fibrate-induced mechanisms underlying normalisation of the atherogenic LDL profile, and which involve both the intravascular metabolism of LDL, as well as its catabolism by the cellular LDL receptor pathway, will be outlined.

Finally, subgroup analyses of data from the NLHLBI-II, STARS and CLAS regression studies indicate that the greatest arteriographic benefit occurred in CAD patients when levels of small, dense LDL were significantly reduced (Circulation, 1994, 90: 1056–1069). Atherogenic dense LDL are therefore of major impact on vascular disease.

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To what LDL level should we treat the CHD patient?

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Observational population studies indicate a curvilinear risk function for total and LDL cholesterol and the risk for coronary heart disease (CHD). This function can be widened to lower plasma cholesterol levels by the inclusion of rural Chinese populations and to higher levels by including cases with familial hypercholesterolemia in the risk equation. The lowest CHD risk is seen in the lowest rural Chinese cohort and the highest in homozygous familial hypercholesterolemia. The hypothesis is that cholesterol lowering treatment in primary and secondary prevention moves the risk of the patient along this observational curvilinear risk function. This means that for each percent decrease in cholesterol at high ranges the diminution in absolute risk is greater than for each percent cholesterol lowering achieved at lower baseline cholesterol levels. In the Scandinavian Simvastatin Survival Study (4S), in which baseline cholesterol levels were between 5.5 and 8.0 mmol/l, analysis of the relation of LDLcholesterol and other lipids during treatment to outcome was made in several ways. The lowest risk of a new event was achieved in those who had the lowest LDL cholesterol during treatment. A curvilinear function between LDL cholesterol levels during treatment and the risk for a new event was demonstrated. Thus no threshold level of neither LDL cholesterol nor LDL/HDL cholesterol ratio could be detected under which further lowering was of no preventive effect. In conclusion 4S data suggest that additional benefit from lipid intervention in CHD patients could be obtained by more aggressive treatment than the average effect obtained in 4S.

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Direct vascular effects of HMG-CoA reductase inhibitors

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Increasing knowledge of the pathogenesis of atherosclerosis suggests that future strategies for the prevention of the coronary artery disease will probably include both corrections of the plasma lipid profile and direct pharmacological control of the processes occurring in the arterial wall. Of these processes, migration and proliferation of smooth muscle cells (SMC), together with lipid deposition, play a pivotal role and are predominant features of atherogenesis. Mevalonate and other intermediates of cholesterol biosynthesis (isoprenoids) are essential for these processes, hence it is conceivable that HMG-CoA reductase inhibitors (statins) can directly affect major events occurring in arterial wall during atherogenesis, independently of their lipid-lowering effect. Recently, we provided "in vitro" and "in vivo" evidence that fluvastatin (F) and simvastatin (S), but not pravastatin (P), dose-dependently decreased SMC migration and proliferation. These cellular effects were prevented by the addition of mevalonate and by its derivatives farnesol and geranylgeraniol confirming the specific role of isoprenoid metabolites, probably through a prenylated protein(s), in regulating these cellular events. The inhibitory effect of statins on SMC proliferation are due primarily to a cell cycle specific action at the G1 phase through a modulation of the level of cyclins and cyclin-dependent kinase inhibitors (p21, p27), nuclear proteins playing a crucial role in the control of the cell cycle. The ability of F to interfere with arterial SMC proliferation "in vitro" at therapeutic concentrations (0.1–1 μM) prompted investigation of the pharmacological activity of sera from 10 patients treated with 40 mg uid F on the proliferation of cultured human SMC, and their effects on cholesterol synthesis. The more hydrophilic P (40 mg uid), with a lipid lowering effect similar to F, but without "in vitro" effect on SMC proliferation, provides a unique control in assessing this non-lipid related effect of F. Treatment of patients with type IIa hypercholesterolemia with F or P for 6 days resulted in a similar effect on plasma lipids and lipoprotein concentrations. However, the addition of 15% whole-blood sera from patients treated with F to the culture medium caused an inhibition (43%; $p < 0.01$) of cholesterol synthesis in SMC, that mirrored the pharmacokinetic profile of F. When SMC proliferation was investigated, a significant inhibition of cell growth (28% decrease; $p < 0.01$) was detected with sera obtained 6 h after the last dose. No effect was observed on either SMC proliferation or cholesterol biosynthesis with sera from patients treated with P. Concerning lipid deposition, S and F also inhibited cholesterol esterification and cellular deposition of cholesteryl esters induced by acetylated LDL in cultured macrophages. This effect was fully prevented by the addition of mevalonate or geranylgeraniol.
Taken together, these results suggest that beyond their effect on plasma lipids, HMG-CoA reductase inhibitors exert a direct antiatherosclerotic effect on the arterial wall, probably through local inhibition of isoprenoids biosynthesis, that could translate into more significant prevention of cardiovascular disease.

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Statins and CHD Prevention

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The statins, since their introduction into clinical practice almost a decade ago, have captured and expanded the cholesterol-lowering drug market. Until then, most lipid lowering agents available were either modest in their effectiveness or so associated with such side effects as to make them unacceptable to patients. The potency of the statins, though obviously beneficial in clinical terms, was in the first instance viewed by many with a degree of concern since they act primarily by targeting the rate limiting step in the important metabolic cascade leading to cholesterol biosynthesis in hepatocytes. Their competitive inhibition of hepatocyte 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMG CoA reductase) limits the availability of cholesterol within these cells and, in consequence, upregulates LDL receptors on their cytoplasmic membrane. This leads to accelerated plasma clearance not only of LDL but also of its precursors VLDL and IDL. Therefore, circulating LDL levels fall, partly because of an increase in catabolism and also because LDL precursor availability is curtailed.
The publication of a series of statin-based intervention trials like the West of Scotland Study (WOSCOPS), the Scandinavian Simvastatin Survival Study (4S) and the Cholesterol and Recurrent Events (CARE) study have established the safety and efficacy of the drugs. All showed that statin treatment reduced the risk of cardiovascular morbidity and mortality without increasing the risk of non-cardiovascular events. These findings dispel our concerns over whether to treat raised cholesterol levels and change the point of discussion to when and how.

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Clinical importance of new insights into the mechanism of activation of fibrates

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Treatment with fibrates, a widely used class of lipid modifying agents, results in a substantial decrease in plasma triglycerides, and is usually associated with a moderate decrease in low density lipoprotein (LDL) cholesterol and an increase in high density lipoprotein (HDL) cholesterol concentrations. Fibrates induce marked changes in the physicochemical characteristics of lipoprotein particles, which include a reduction in the proportion of the atherogenic dense LDL resulting in increased resistance of LDL to oxidation. These changes in lipoproteins are correlated to alterations in their apolipoprotein (apo) content, such as decreased apo B, apo C-III and apo E levels in triglyceride-rich lipoproteins and increased apo A-I and apo A-II in HDL. Recent investigations indicate that the effects of fibrates are, at least in part, mediated through alterations in transcription of genes encoding for proteins which control lipoprotein metabolism. Fibrates activate specific transcription factors belonging to the nuclear hormone receptor superfamily, termed peroxisome proliferator activated receptors (PPARs). PPARs bind as heterodimers with another nuclear receptor, the retinoic receptor RXR, to specific response elements termed peroxisome proliferator response elements (PPREs) and alter the transcription rate of target genes. Among the different PPARs, the PPARα form mediates fibrates action on HDL-C levels via transcriptional induction of synthesis of the major HDL apolipoproteins, apo A-I and apo A-II. The hypotriglyceridemic action of fibrates also involves PPARs and is in part due to increased LDL-mediated lipolysis, which is in part linked to lowered hepatic apo C-III production. Furthermore, a reduction in fatty acid and triglyceride synthesis and a decrease in very low density lipoprotein (VLDL) production have been documented. In conclusion, both enhanced catabolism of triglyceride rich particles as well as reduced secretion of VLDL underlie the hypotriglyceridemic effect of fibrates, whereas their effect on HDL metabolism is associated with changes in HDL apolipoprotein expression. It is suggested that PPARs are key messengers responsible for the translation of fibrate activity into changes in gene expression. It remains to be shown whether other, yet unidentified molecular mechanisms are also involved.

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Evidence based approach for the management of mixed hyperlipidaemia

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When we consider a plasma lipid profile that will contribute to increased coronary heart disease (CHD) risk we think of a raised total cholesterol (C) level and more specifically a raised LDL-C level. However, it is also important to consider the impact of an increased triglyceride (TG) and reduced HDL-C concentration.
There is an abundance of observational and interventional evidence that leads us to conclude a cause and effect relationship between dyslipidaemia and CHD risk. Most emphasis has been placed on LDL-C as the principal atherogenic lipid. However, the plasma HDL-C and the TG are also often abnormal in patients presenting with CHD.
There is a large body of evidence from multiple trials confirming that LDL-C lowering results in a clinically significant reduction in CHD risk. Most recently trials conducted with drugs that primarily lower LDL-C were the Scandinavian Simvastatin Survival Study and the West of Scotland Study. Both trials used statins and lowered LDL-C by 35% (4S) and 26% (WOSS) resulting in significant falls in coronary events.
Further analyses of the Helsinki Heart Study (HHS) data have provided additional detail on the relationship between other lipid changes and incidence of CHD. The success of gemfibrozil, a fibrate, in reducing CHD events was related not only to its ability to lower LDL-C but also to its HDL-C raising effect. Using subgroup analysis a high-risk group of subjects were identified. These were found to have an LDL/HDL-C ratio > 5 and plasma TG > 2.3 mmol/l. This group profited most from gemfibrozil therapy in the HHS with a 71% lower incidence of CHD events than the corresponding placebo subgroup. We may hypothesize that if a similar intervention trial were designed only to include this high risk group in sufficient numbers, the clinical efficacy of the fibrate in reducing CHD morbidity would be dramatic.
The publication of a major fibrate regression trial (BECAIT) gives us the opportunity to compare the levels of regression associated with different drug classes. The treatment effect on minimum lumen diameter in mm was 0.13 on the fibrate but only 0.06 or 0.08 on the statins. The statins produced much greater changes in total and LDL-C but it was the fibrate that had the most marked effect on the TG level.
In practice, the overall CHD risk of patients should be reduced by a co-ordinated approach to management of all the correctable risk factors. As part of this approach the lipid profile should be assessed and normalised initially through the instigation of lifestyle changes and if necessary the adjunctive use of lipid regulating drugs.
When we decide to use a lipid regulating drug the choice of agent must be made carefully. If a patient has isolated hypercholesterolaemia, the statins are clearly the drugs of first choice in the absence of any contraindication. If, however, the patient has a low HDL-C and a raised plasma TG either alone or in conjunction with a raised LDL-C then a fibrate may be most appropriate.

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Identifying the high risk Patient for lipid lowering therapy

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Clinical trials with angiographic and clinical end points have convinced most clinicians that lipid lowering therapy is worth while in most patients with manifest coronary disease. The area of uncertainty is now in primary prevention: how do we identify the apparently healthy person at high relative and, in particular, absolute risk of getting the disease?
Characteristics of coronary atherosclerosis are the long dormant phase and the unpredictability of myocardial infarction and coronary death. It would therefore be important to detect the disease preclinically, but no current method, including exercise electrocardiography, myocardial scintigraphy, coronary angiography, intravascular ultrasound, MR scanning or CT scanning, is entirely satisfactory.
Pending technological developments, we are still consigned to assessing risk based on classical risk factors. The European Atherosclerosis Society, the European Society of Cardiology and the European Society of Hypertension have jointly provided a practical set of guidelines with an assessment of absolute risk based on age, sex, smoking, systolic blood pressure and serum cholesterol. Published in 1994, the guidelines already need revision to reflect more recent insights.

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Cellular and molecular aspects of atherosclerosis: new and old theories

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Atherosclerosis represents the culmination of a chronic inflammatory process involving smooth muscle cells and macrophages, accompanied by a fibroproliferative process. The initial lesion of human atherosclerosis, the fatty streak, begins as a result of monocyte adhesion on an "activated" endothelial cell surface, and then migration of monocytes into the sub-endothelial space, the intima. These cells, once differentiated into macrophages, as well as smooth muscle cells, proliferate and become the progenitors for the atherosclerotic "foam cell" in the presence of a lipid-enriched diet.
There is significant evidence supporting the "response-to-injury" hypothesis for the pathogenesis of atherosclerosis. Based on animal and human data, conclusions have now been reached that viruses may behave as active participants in those processes related to vascular endothelial cell injury, intimal lipid accumulation, and activation of the coagulation cascade on the endothelial cell surface. The culmination of these processes can predispose the vessel wall to be a thrombo-arteritis.
New data will be presented which document the role of herpesviruses in the pathogenesis of atherosclerosis. This information will be presented in the context of other theories for the arteriopathy in an attempt to understand the complex nature of atherogenesis which involves cell adhesion and proliferation, cholesterol accumulation, and thrombogenesis.
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Δ5 olefinic acids extracted from pine tree seeds decrease serum triglyceride levels in rats.

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The goal of the present study was to assess the lipid lowering potential of vegetal oils extracted from the *Pinus pinaster* and *Pinus koraiensis* seeds. These seeds contain particular unsaturated fatty acids (named Δ5 olefinic acids): the *all cis* 5,9,12 18:3 and *all cis* 5, 11,14 20:3 acids. The amount of these fatty acids are respectively 7.1% and 7.1% of total fatty acids in *Pinus pinaster* and 14.9% and 0.9% in *Pinus koraiensis*. These fatty acids have 18 or 20 carbons, like eicosapentaenoic and docosahexaenoic faty acids they have a double bond at position Δ5, two double bonds, however, are separated by more than one carbon. The effects of Δ5 olefinic acids on lipid and lipoprotein parameters were compared to those of oleic acid. Four groups of six rats were fed one of the four following regimes for four weeks: 1/ *Pinus pinaster* seed oil, 2/ *Pinus koraiensis* seed oil and 3/ and 4/ two oil mixtures established to contain the same fatty acids as the corresponding pine seed oil except for Δ5 olefinic acids that were replaced by oleic acid. The oils (5%) were included in a fat free diet. As compared to its control, *Pinus pinaster* oil decrease triglycerides (TG) by 30% ($p < 0.05$), VLDL-TG by 40% ($p < 0.05$), VLDL-cholesterol by 33% ($p < 0.01$), VLDL-phospholipids by 21% ($p < 0.05$) and apo A-II by 18% ($p < 0.05$). Although *Pinus koraiensis* decrease serum TG by 16% and VLDL-TG by 21% these changes did not reach the level of statistical significance. Gel permeation chromatography and non-denaturating polyacrylamide gel electrophoresis showed a tendency to a shift of HDL toward larger particles in rats treated with *Pinus pinaster* and *Pinus koraiensis* oils relative to their control oils. These changes were not associated with significant alterations in HDL lipid composition. Hepatic cholesterol esterification rate was measured by the incorporation of ^{14}C oleic acid to cholesterol esters. There was no statistically significant difference in the rate of cholesterol esterification among diet groups. In conclusion, compared to oleic acid Δ5 olefinic acids extracted from *Pinus pinaster* and *Pinus koraiensis* seeds have a triglycerides lowering effect in rats. *Pinus pinaster* oil appears to be more potent than *Pinus koraiensis* oil suggesting that *all cis*-5,11,14 20:3 acid has a greater lipid lowering effect than *all cis* 5,9,12 18:3 acid.

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Effect of dietary fish oil or sunflower oil on plasma lipoproteins and hepatic gene expression in the hamster

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An increased dietary consumption of fish oils reduces the occurrence of coronary artery disease. Although this probably involves several mechanisms, one effect of an increased consumption of fish oil is a fall in plasma triacylglycerol (TAG) in humans and experimental animals. This has led to the idea that fish oil fatty acids lower VLDL secretion by the liver and hence reduce the risk of atherosclerosis. To investigate this, we have supplimented the diets of hamsters with 10% fish-oil or 10% sunflower oil and examined blood lipoproteins, VLDL secretion and the mRNA levels of genes involved in apolipoprotein B metabolism. Hamsters were fed 1.5 ml of either fish oil (Maxepa) or sunflower oil by daily gavage. Blood samples were taken at weekly intervals. The lipids were analysed and the lipoprotein classes separated on iodixanol gradients and by agarose electrophoresis. In fish oil fed animals, plasma TAG fell by 25% over a three week period. There was no significant change in the TAG levels of either chow fed animals or sunflower oil fed animals nor in the cholesterol levels of the three groups of animals. Plasma VLDL were considerably reduced and there was a small increase in LDL and HDL in the fish-oil fed animals. In the sunflower oil fed and chow fed animals, there was no significant change in the plasma lipoproteins. Consistent with these results from *in vivo* experiments, secretion of VLDL by hepatocytes isolated from fish-oil fed hamsters was considerably reduced compared with hepatocytes from chow-fed and sunflower-oil fed animals. The fall in plasma VLDL in fish-oil fed hamsters was not accompanied by a fall in LDL. To determine whether this is due to decreased LDL uptake we determined the mRNA levels for the LDL receptor (LDL-R) in liver from hamsters fed fish-oils, sunflower oil or chow for three weeks using the mRNA protection assay. The mRNA for apo-B, HMG-CoA reductase and microsomal triacylglycerol transfer protein (MTP) were also measured in the same liver samples to examine possible effects on other candidate proteins involved in apo-B metabolism (Table 1).

Table 1 mRNA levels (attomoles/μg liver ± SD (n = 4) normalised to 10 μg polyA RNA per μg of liver

mRNA	Fish-oil fed	Sunflower oil fed	Chow-fed
HMG-CoA reductase	2.10 ± 0.34	3.61 ± 0.82	5.30 ± 0.77
LDL-R	1.90 ± 0.20	4.12 ± 0.65	4.50 ± 1.27
MTP	17.54 ± 2.81	19.32 ± 4.34	20.23 ± 3.34
Apo-B	39.5 ± 4.34	33.85 ± 3.33	56.72 ± 25.56

Dietary fish-oils reduced the levels of MRNA for the LDL-R by approximately 60% while dietary sunflower oil had no significant effect, mRNA for HMG-CoA reductase was reduced 60% by feeding fish-oils and 20% by feeding sunflower oils. These results suggest that there may be a complex metabolic relationship between apo-B synthesis and uptake by the liver which is perturbed by feeding fish-oils

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Cerivastatin: High enzyme affinity and active metabolites contribute to its high pharmacological activity.

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HMG-CoA reductase inhibitors have proven their effectiveness in primary and secondary prevention of coronary artery disease. For the treatment of hypercholesterolaemia, vastatins are used in daily dosages between 10 and 80 mg. Significantly lower doses in the µg-range are thought to be used with the new synthetic and enantiomerically pure HMG-CoA reductase inhibitor cerivastatin with only 0.1 to 0.3 mg. The differences in the therapeutic doses are reflected by the IC_{50}-values determined in enzyme inhibition tests. In isolated enzyme preparations using the native ribosomal fraction from rat liver and a NADPH-regenerating system IC_{50}-values of 1.1 x 10^{-9} M for cerivastatin1 66 x 10^{-9} M for simvastatin, 77 x 10^{-9} M for lovastatin and 176 x 10^{-9} M for pravastatin were determined. In addition to these differences, cerivastatin showed a favourable liver selectivity. Otherwise than in animals the metabolism of cerivastatin in man results in the formation of cerivastatin metabolites which have shown to be highly active inhibitors not only in vitro but also in vivo. The demethylated and hydroxylated metabolites BAY 17–5111 and BAY 19–3103 inhibited the HMG-CoA reductase isolated from rat liver with the same potency as the parent compound cerivastatin. Corresponding pharmacological activity was observed in vivo. Both metabolites inhibited ^{14}C-cholesterol synthesis from ^{14}C-acetate in rat liver with ED_{50}-values between 0.001 and 0.002 mg/kg body weight which is similar to cerivastatin (ED_{50}: 0.002 mg/kg). The strong inhibitory activity of these metabolites might also contribute to the high pharmacological activity of cerivastatin and to its ultra-low dose in man.

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Using of peripheral blood lymphocytes: early diagnosis a different type of hyperlipidemia.

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1. Purpose. Though a correlation between the incidence of ischemic heart disease and plasma level of `atherogenic' cholesterol has been demonstrated in a number of extended population studies, the mechanism underlying this correlation still remains unclear. This study is an attempt to compare the changes in the cholesterol content of peripheral blood lymphocytes with plasma concentrations of total cholesterol, triglycerides and cholesterol derived from low density lipoproteins (LDL) and high density lipoproteins (HDL).
2. Materials and methods. Variability of cholesterol level inside lymphocytes (CLL) have been compared with the concentration of total cholesterol, triglyceride, LDL and HDL of blood plasma among normal donors and hyperlipidemic patients and their relatives.
3. Results. Persons with elevated CLL was found among normal donors and hyperlipidemic patients with the occurrence rate of 4–5%. No significant correlation was followed between CLL and the level of total cholesterol, triglyceride, LDL and HDL blood plasma level among total observed population. However it was found that all patients with familial hypercholesterolemia (hetrozygous and homozygous form) have very low level cholesterol inside lymphocytes and very high level cholesterol of plasma.
4. Conclusion. We suppose that this method may be used for the earlier diagnosis of familial hypercholesterolemia.

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The effects of garlic powder supplementation on lipoprotein oxidation in mild hyperlipidaemia.

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The reported lipid lowering and antioxidant effects of Garlic (*Allium sativum L*) were investigated in this double blind randomised six month parallel trial. Plasma lipids, Apolipoproteins Al,B, and Lipoprotein (a) were determined along with copper ion induced oxidation of isolated LDL (Lag Time) and antibodies to oxidised LDL (Abs.ox-LDL). Analysis was restricted to subjects with > 75% compliance. Subjects (n = 20) and controls (n = 11) having Total Cholesterol 6.08.5mmol/L received 900mg / day of dried garlic (standardised to 0.6% Allicin). Lag times increased significantly in both groups, Garlic p = 0.013, Placebo p = 0.0002, differences between groups were not significant. The increase in Lag Time was not accompanied by any significant alterations in the levels of Abs.ox-LDL. A nonsignificant decrease in Total Cholesterol was observed in the Garlic group, the Placebo group showed a significant increase in plasma HDL Cholesterol levels (p = 0.0005). None of the other analytes showed any changes. The increase in Lag Time may be due to a change in diet between the pre and post samples and possibly with seasonal variations in Vitamin E and other antioxidants. The previously reported lipid lowering and antioxidant effects of garlic are not supported by this study.

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ACE gene polymorphism in old hypertensive or normotensive subjects with angiographically proven coronary heart disease.

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Background: It has been suggested that the insertion/deletion polymorphism (I/D) of the ACE gene is an independent risk factor for coronary heart disease (CHD). Little is known about influence of age on I/D ACE allele frequency in hypertensive or normotensive CHD patients.
Material and methods: The study was performed in 100 patients with CHD confirmed by coronarography, all were older than 65 years of age (mean age 69 ± 4 years, 67 males and 33 females). 54% of subjects were hypertensive. The polymerase chain reaction and agarose gel electrophoresis was used to determine the ACE I/D genotype.
Results: The observed ACE genotype frequencies in our study group DD (30%), ID (51%) and II (19%) were in Hardy-Weinberg equilibrium (p 0.95). The ACE genotype in old subjects with CHD was not significantly associated with either myocardial infarction in history or arterial hypertension. The odds ratios associated with D allele was 1.25 (p = 0.5) for myocardial infarction and 1.58 (p = 0.17) for arterial hypertension. In addition there was no association of the I/D polymorphism and the number of affected coronary arteries determined by angiography (single vessel disease vs. multiple vessel disease, odds ratio 1.11; p 0.79) in the total study group and in hypertensive subject odds ratio was 0.91 (p = 0.48). However the hypertension status was correlated with the number of significant coronary artery stenosis (hypertensives vs. normotensives odds ratio 1.21 p < 0.01). Segregation of the ACE genotypes was independent of common risk factors: age, body mass index, plasma lipoproteins, triglicerydes, diabetes and smoking habits.
Conclusion: The D allele of the ACE gene studied in older age group is not associated with angoigraphically proven coronary heart disease or hypertension status however hypertension itself correlates with the number of affected coronary arteries.

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Short-term Effects of Dietary Counselling in Endogenous Hypertriglyceridemia

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Introduction: Endogenous hypertriglyceridemia (HTG) is a multifactorial lipid disorder, characterized by elevated levels of very low density lipoproteins (VLDL) and decreased concentrations of high density lipoprotein cholesterol (HDL-C). Environmental factors are considered to play an important role in the expression of these lipid abnormalities. Accordingly, dietary and life-style measures are first-line strategy in these patients.
Aim of the study: This study was designed to investigate the short-term effects of dietary counselling in a population of patients with endogenous HTG (Fredickson type IV and V hyperlipoproteinemia).
Materials and methods: 43 patients with endogenous HTG participated in this dietary interventoin study between 1988 and 1996. Both at intake and 12 weeks after dietary counselling, blood samples were taken and dietary intake was assessed by a trained dietician. By means of a 24-h food recall and a computerized food table, the nutrient content of the diet was calculated. Dietary guidelines were 1) Total fat < 30% of energy intake, 2) Saturated fat < 10% of energy intake, 3) Dietary cholesterol < 300 mg/d and calorie reduction in case of obesity.
Results and discussion: The calculated energy intake decreased from 2142 to 1660 kcal/d (−22%, $p < 0.001$), carbohydrate intake decreased from 231 to 202 g/d (−13%, $p < 0.01$), total fat intake dropped from 78 to 49 g/d (−37%, $p < 0.001$), the ratio saturated/unsaturated fat ameliorated from 0.72 to 0.60 (−17%, $p < 0.001$), and alcohol intake decreased from 13 to 5 g/d (−60%, $p < 0.001$). Body mass index improved slightly from 28.3 to 27.6 kg/m² (−2%, $p < 0.001$). Changes in plasma lipids and lipoproteins are shown in the table.

	Chol	TG	VLDL-C	VLDL-TG	LDL-C	HDL-C
			mmol/l			
Before	9.3	16.8	6.2	16.5	2.69	0.62
After	7.9	11.3	4.0	9.4	3.03	0.68
Change (%)	−14%**	−32%**	−35%**	−43%**	+13%	+10%

Regression analysis was performed to determine which dietary changes correlate with TG reduction. Only weight reductoin ($r = 0.36$, $p < 0.05$) and limitation of alcohol intake ($r = 0.56$, $p < 0.001$) showed a correlation with TG reduction. In conclusion, short-term dietary counselling is effective in reducing VLDL concentrations in endogenous HTG subjects. Unlike (isocaloric) shifts in dietary compounds towards a more 'healthy', balanced diet, weight loss and reduction of alcohol intake appear to be most effective in improving lipid levels in endogenous HTG.

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Familial hypercholesterolemia and familial defective apolipoprotein B in Belgium

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From a cohort of hyperlipidemic patients who attended the lipid clinics of Gent and Jolimont, 202 unrelated patients of Belgian descent (115 Flemish speaking and 87 French speaking Belgians) were selected on the basis of a total cholesterol level greater than 300 mg/dl and a dominant pattern of inheritance of hypercholesterolemia in their families. Classical genetic analysis (denaturating gradient gel electrophoresis, sequencing and direct test) were applied to look for LDL receptor point mutations and the apoB3500 mutation.
Results. In the LDL receptor gene, 18 different point mutations have been so far discovered: E10X (1,0), 83A (0,7), E92X (0,1), C122X (123,3), ∇TGTG442 (0,1), S223Y (0,1), C292W (2,0), E256K (2,0), R329X (0,1), T413R (1,1), G571E (1,1), D601Y (0,1), P640L (0,3), Q639X (0,1), P664L(1,4), T7051 (0,2) and 2 splice mutations: G→A313 + 1 (0,7), Da1186 − 2 (0,2).
Most of these mutations have not been so far described in other parts of the world. The distribution of mutations was different in 2 parts of the country. Only four of these mutations were found in both parts of the country. C122X appeared very common in Belgium specially in Wallonia (12 families). The G→A313 + 1 already described in Denmark and Netherlands appeared very common only in Flanders (7 families). ApoB3500 was only observed in 8 patients of cohort. This may suggest that FDB is associated with a less higher cholesterol level or is associated less common in Belgium than in other European countries.
We concluded that FH is the most common cause of severe hypercholesterolemia in Belgium. The difference in LDL receptor mutation between the Northern and the Southern part of Belgium reflects a difference in genetic background. The specific geographic distribution suggests that in the future, molecular diagnosis of FH in Belgium may be oriented by the origin of the family.

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GHHAINAUT PROJECT: Identification of genetic hypercholesterolemia in Hainaut.

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Familial hypercholesterolemia (FH) and familial defective apolipoprotein B (FDB) share a common pattern of severe hypercholesterolemia (HC), early coronary disease (CHD) and sometimes tendon xanthomas (TX). GHHainaut is a project aimed to promote identification of FH/FDB in the Province de Hainaut by general practitioners (GP) through a molecular diagnosis service.
Design. In 1996, 176 GP were asked to collect clinical data and blood samples in families with evidence of inherited HC ([TC] > 300 mg/dl). Classical genetic screening for LDL receptor mutations and FDB were conducted in one adult index case of each family (Phase I). In phase II, the GP were informed of the results, asked to give information about cholesterol screening and hypolipemic therapy in the members of the families and requested to perform a Achille tendon (AT) sonography.
Preliminary results. Phase I. 207 blood samples from 156 families were collected by 77 GP (43% participation). TC measured in the index cases was 396 ± 61 mg/dl. 92% of the 156 families showed at least one first degree relative with early CHD but TX were identified in only 17% of these families. So far genetic analysis (apB3500 and LDL-R exons 2–6, 10, 14) identified 2 FDB and 24 FH (11 different mutations). TC on these 26 index patients (435 ± 64 mg/dl) and the prevalence fo CHD (25/26) and TX (6/26) in their families were slightly higher than in the whole cohort. Phase II. Amongst the 26 ascertained FH/FDB index cases, only 16 index cases were receiving a hypolipemic treatment and amongst the treated patients, only 4 had a TC below 300 mg/dl. Only 2 families were screened for HC in the children above 12. The performance of AT sonography in the 26 index cases resulted in the discovery of TX in 10 more patients.
Conclusions. These preliminary data advocate the need for a better education in the recognition and treatment of the FH/FDB patients.
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Measurement of LDL receptor activity in T-lymphocytes amongst a genetically ascertained FH population

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We had previously described a method to measure the activity of the LDL receptor (LDL-R) in the T lymphocytes that was used to differentiate FH and non FH patients amongst a population of primary hypercholesterolemia. We now assess the method in genetically ascertained FH patients.
Methods. Peripherical blood mononuclear cells (PBMC) preincubated for 42 hours in delipidated medium were incubated first with dil-LDL for 30 min. and then with FITC-anitCD3+ for 10 min.. Uptake of dil-LDL in CD3+lymphocytes (T-Lc) was analyzed specifically using flux cytometry and expressed as the log of the mean dil-fluorescence (logF).
Subjects. 33 normocholesterolemic individuals were the controls (group 1). The FH population were 18 patients (group 2), each with a different mutation of the LDL-R. A group of 13 FH patients (group 3) carrying the same mutation C122X, a severe class 1 receptor defect was specifically analyzed.
Results. Log(F) was 1.28 ± 0.21 in group 1 and 0.86 ± 0.23 in group 2. Three FH patients (all women) had a very high value (above 1.28). Two normal individuals (young male) had very low values (below 0.86) at repeated tests. Based on these results, we set the cut off value of logF at 1.07 (the medium of between the means of group 1 and 2). The sensitivity and specificity were then estimated to 85%. When studying the population of patients with the severe $C122X^+$ FH (group 3), values tended to be lower and had a narrower distribution (0.69 ± 0.12).
Conclusions. We confirmed the usefulness of this method in the diagnosis of FH as specificity and sensitivity appeared high.

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Cerivastatin—a new HMG-CoA reductase inhibitor—in combination with the antacid Maalox®: an investigation of the safety and pharmacokinetics

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Cerivastatin is a novel, synthetic, potent and highly selective inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase that effectively reduces serum cholesterol levels at very low doses. As cerivastatin possesses an acid function, the possible influence of pH-changes induced by antacid administration on cerivastatin absorption was of interest. In a phase I, prospective, controlled, randomised, crossover study in 8 healthy male volunteers (aged 18-45 years), the effects of concomitant administration of cerivastatin and Maalox® 70 (antacid based on magnesium-aluminium hydroxide) were studied. Subjects received a single oral dose of 0.2mg cerivastatin with or without concomitant administration of 10 mL Maalox® 70. A 7-day washout period was allowed between administrations. Cerivastatin was well tolerated and no clinically relevant adverse events or changes in laboratory parameters were observed. Vital signs and ECG remained unchanged.

Cerivastatin	AUC	C_{max}	t_{max}*	t1/2
(geom. means/SD)	[µg•h/L]	[µg/L]	[h]	[h]
plus Maalox® 70	10.44/1.37	1.77/1.29	3.0	2.67/1.24
alone	11.38/1.46	1.99/1.48	3.0	2.69/1.29

*median

Statistical analysis showed that a pharmacokinetic interaction between the antacid Maalox® 70 and cerivastatin did not occur. The concomitant administration was safe and well tolerated.

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Increased activity of PAF-acetylhydrolase in low density lipoprotein subfractions induces enchanced lysophosphatidylcholine production in patients with heterozygous familial hypercholesterolemia

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Patients with heterozygous familial hypercholesterolemia (FH) have elevated plasma concentrations of low density lipoprotein (LDL) and develop premature atherosclerosis. There is increasing evidence that oxidative modification of LDL plays an important role in the pathogenesis of atherosclerosis and the LDL-associated platelet-activating factor acetylhydrolase (PAF-AH) seems to play a key role in the LDL oxidation by hydrolyzing the oxidized phospholipids of phosphatidylcholine (PC) and producing lysophosphatidylcholine (Lyso-PC). We measured the total serum and HDL levels of PAF-AH activity and we studied the distribution of PAF-AH activity among 3 LDL subfractions isolated by gradient ultracentrifugation in 15 patients with heterozygous FH and 13 normolipidemic controls. We also determined the Lyso-PC production in each LDL subfraction during Cu^{2+} -induced oxidation *in vitro*. The total serum PAF-AH activity in heterozygotes FH patients was significantly higher compared to controls, whereas the HDL-associated PAF-AH activity, expressed as percent of total serum PAF-AH activity, was significantly lower in the FH patients as compared to controls (13.9 ± 6.6% versus 30.6 ± 4.4%, respectively) ($P < 0.001$). Among the LDL subfractions, the PAF-AH activity in both normolipidemic controls and FH patients was significantly higher in LDL3 subfraction (33.1 ± 4.8 nmol/mg of protein/min and 53.4 ± 11.5 nmol/mg of protein/min, respectively), compared to LDL2 (18.6 ± 5.3 nmol/mg of protein/min and 26.8 ± 10.4 nmol/mg of protein/min, respectively) ($P < 0.0001$) and to LDL1 (5.1 ± 1.5 nmol/mg of protein per min and 7.8 ± 2.6 nmol/mg of protein per min, respectively) ($P < 0.0001$). Additionally, the enzyme activity in each LDL subfraction of the heterozygotes FH patients was significantly higher compared to controls, $p < 0.02$ for LDL1, $P < 0.03$ for LDL2 and $P < 0.0001$ for LDL3. No difference was observed in the susceptibility to oxidation of each LDL subfraction among the heterozygotes FH patients and the normolipidemic controls. During oxidation, the PAF-AH activity was decreased whereas the Lyso-PC levels were significantly increased in all subfractions of both groups. The Lyso-PC/Sphingomyelin molar ration in each LDL subfraction of the FH patients 3h after the onset of the oxidation was significantly higher compared to controls, 0.38 ± 0.05 and 0.27 ± 0.04 respectively for LDL1 ($P < 0.006$), 0.47 ± 0.08 and 0.39 ± 0.03 respectively for LDL2 ($P < 0.04$), 0.55 ± 0.11 and 0.42 ± 0.06 respectively for LDL3 ($P < 0.02$). Our results show that heterozygotes FH patients exhibit higher PAF-AH activity in all LDL subfractions compared to controls resulting in higher Lyso-PC production during oxidation, despite the absence of difference in the susceptibility to oxidation of each subfraction between the two groups. This phenomenon in combination with the diminished antioxidant and antiatherogenic capability of HDL due to the lower HDLcholesterol levels as compared to LDL-cholesterol levels and the relatively lower HDLassociated PAF-AH activity, could contribute to the higher atherogenicity and incidence of coronary artery disease observed in these patients.

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Phase I study to investigate the effects of cimetidine on the pharmacokinetics of cerivastatin—a new HMG-CoA reductase inhibitor

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In a prospective, single-centre, randomised, crossover study in 8 healthy male subjects the effects of oral cimetidine (Tagamet®) on the pharmacokinetics of cerivastatin, a novel, synthetic, potent and highly selective 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, were studied. A total of 8 healthy males (aged 18–45 years) received cerivastatin with and without cimetidine. Subjects were pre-treated for 3 days with 2 x 400mg cimetidine followed by 1 x 400mg cimetidine plus a single oral administration of 0.2mg cerivastatin or received a single dose of 0.2mg cerivastatin on the profile day. Cerivastatin plasma concentrations were measured by a specific and sensitive HPLC assay and a RIA was used to quantify cerivastatin and its immunoactive metabolites. There were no adverse events associated with cerivastatin and no clinically relevant changes in laboratory parametres or vital signs were observed.

Cerivastatin	AUC	C_{max}	t_{max}*	$t_{1/2}$
(geom. means/SD)	[µg•h/L]	[µg/L]	[h]	[h]
plus cimetidine	10.09/1.12	2.06/1.19	3.0	1.96/1.18
alone	11.04/1.17	2.18/1.10	3.0	2.13/1.11

*HPLC data **median

Statistical analysis of both HPLC and RIA data showed that a pharmacokinetic interaction between cimetidine and cerivastatin did not occur. The concomitant administration was safe and well tolerated.

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Deletion polymorphism of the ACE gene is related to history of myocardial infarction but negatively correlates with history of hypertension in patients with coronary heart disease below 50 years of age.

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Background: It has been suggested that the insertion/deletion polymorphism (I/D) of the ACE gene is an independent risk factor for coronary heart disease (CHD). Little is known about I/D ACE allele frequency relation to hypertension or myocardial infarction in history of patients with CHD in different age groups.
Material and methods: The study was performed in patients with CHD confirmed by coronarography in two age groups. 1–100 subjects older than 65 years of age (mean age 69 ± 4 years, 67 males and 33 females) 54% were hypertensive and 50% survived myocardial infarction (MI). 2–99 subjects younger than 50 years of age (mean age 44 ± 4 years, 95 males and 4 females) 35% were hypertensive and 73% survived MI. The polymerase chain reaction and agarose gel electrophoresis was used to determine the ACE I/D genotype.
Results: The observed ACE genotype frequencies in our study groups were 1; DD (30%), ID (51%), II (19%) and 2; DD (36%), ID (43%), 11 (21%) respectively (in both groups in agreement with the Hardy-Weinberg equilibrium). There were no significant differences in allele frequencies between studied groups. The DD genotype was more related to the history of MI in younger group in comparison to older patients, the odds ratios DD vs. ID+II were 2.57 ($p = 0.08$) and 0.77 ($p = 0.6$) respectively. The DD genotype in older subjects with CHD was more associated with arterial hypertension in history, odds ratio 2.17 ($p = 0.13$) in comparison to younger patients, odds ratio 0.39 ($p < 0.05$). In addition there was no significant association of the DD vs. ID + II genotypes with the number of affected coronary arteries determined by angiography (single vessel disease vs. multiple vessel disease), odds ratio 1.02 in younger group and 0.7 in older subjects. Segregation of the ACE genotypes was independent of common risk factors: age, body mass index, plasma lipoproteins, triglicerydes, diabetes and smoking habits.
Conclusion: The DD genotype of the ACE gene is more related to myocardial infarction in younger subjects and to hypertension in older patients with coronary heart disease. Age is an important factor in clinical analysis of ACE polymorphism.

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Lipid-lowering therapy: effect on cholesterol efflux from the cells

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It is well known that both Lipanthyl and Endur-acin treatment along with a decrease in the plasma levels of atherogenic lipoproteins can increase the concentration of HDL_{tot} in blood serum that may lead to the stimulation of cholesterol efflux. The aim of present work was to study the ability of sera from hypercholesterolemic subjects to stimulate efflux of cellular cholesterol before and after 3 months treatment with 200 μg/day of Lipanthyl-200M (Laboratoire Fournier, France) or 1.5 g/day of Endur-acin (Endurance Products Co, USA). The human skin fibroblasts as a cell culture system were employed to test the samples of human serum. The ^{3}H-cholesterol efflux was assessed as label appearance in the medium and efflux was measured as the percent ratio of the ^{3}H-cholesterol amount accepted by 10% patients serum to the total amount of cellular ^{3}H-sterols. After 3-months of treatment with Lipanthyl (n = 6) the efflux values were increased as compared with pretreatment data (54.0 ± 2.4 vs 51.3 ± 1.0%, respectively). Simultaneously there was a shift in cholesterol redistribution between intracellular pools: a decrease of free 3H-cholesterol (52. ± 5.9 vs 48.2 ± 4.8% in the control cells) and an increase of the cholesteryl esters (23.4 ± 2.9 vs 25.8 ± 2%).

In the contrast, the incubation of cultured cells with 10% patient serum obtained after Endur-acin treatment did not change cholesterol efflux as well as cholesterol movement in intracellular pools.

Thus, despite the fact that both Lipanthyl and Endur-acin treatment can increase the concentration of HDL_{tot}, the data obtained indicate, that only the sera from Lipanthyl treated patients were able to stimulate cholesterol efflux over initial values.

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Effects of whole soybean products, soy protein and soy oil in hypercholesterolemic individuals

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Dietary substitution of soy protein for animal protein is known to be associated with a reduction of plasma total and LDL cholesterol levels, especially in individuals with hypercholesterolemia. It was hypothesized that this reduction would be greater if whole soybean products (containing both soy protein and soy oil) were substituted for animal products (containing animal protein and fat). To investigate this, the effect of whole soybean products vs. an animal product, milk, was studied in 34 hypercholesterolemic individuals (17 men, 17 women). Using a three-treatment three-period, cross-over design, the subjects received sequentially three types of beverages, each for a period of 4 weeks, with two 2-week washout periods: i) 2% cow's milk products, ii) a combination of skim milk products and soy oil, iii) whole soybean products containing soy protein and 2% soy oil. Daily intakes of calories, protein, carbohydrates and total fat were unaffected by treatment, as assessed by three-day food records. Also, treatment had no significant effect on body mass index, apolipoproteins B and A1 and most of plasma lipids. However, during the whole soybean period, HDL cholesterol increased 9% ($p < 0.04$) and LDL/HDL cholesterol ratio decreased 14% ($p < 0.007$). Similar but less pronounced changes were observed after intake of skim milk/soy oil products but not after intake of 2% milk products. In 24 participants with the highest initial LDL cholesterol and LDL/HDL cholesterol ratio, LDL cholesterol decreased 11% and LDL/HDL cholesterol ratio decreased 19% after the whole soybean period. In the same group, intake of skim milk/soy oil products induced a less pronounced 12% reduction of the LDL/HDL cholesterol ratio. In all subjects, changes in the LDL/HDL cholesterol ratio induced by whole soybean products were correlated negatively with the initial LDL/HDL cholesterol ratio ($r = -0.56$, $p < 0.0007$) and positively with the initial HDL cholesterol ($r = 0.52$, $p < 0.002$). These results demonstrated that in individuals with moderate hypercholesterolemia, intake of whole soybean products can improve the plasma lipid profile and the effect appears to be mostly but not entirely due to soy protein. They also showed that susceptibility to treatment with soybean products is greater in subjects with high initial risk factors of coronary heart disease, elevated LDL and reduced HDL cholesterol levels.

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Haptoglobin polymorphism and peripheral arterial occlusive disease

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Haptoglobin (Hp) is a hemoglobin-binding plasma protein, characterized by a genetic polymorphism with three functionally different phenotypes: Hp 1-1, Hp 2-1, and Hp 2-2. Hp is an antioxidant protecting against the hemoglobin/iron driven oxidation; this protection is less efficient in Hp 2-2. In essential hypertension, atherosclerotic lesions are more common in Hp 2-2 carriers.

As the functional differences between Hp types may influence progression of atherosclerosis, Hp phenotype distribution was investigated in peripheral arterial occlusive disease (PAOD) and compared with those of the reference population. Hp typing was performed by starch gel electrophoresis in 121 patients with PAOD (87 males, 34 females; age: 60 ± 10 yrs). Concomitantly, a reference population (n = 255) was phenotyped. PAOD was defined by values of ankle:brachial systolic blood pressure index < 1 at the walking capacity-limiting leg.

In the reference population, relative Hp type frequencies were 0.16 (Hp 1 - 1; n = 41), 0.48 (Hp 2-1; n = 122), and 0.36 (Hp 2-2; n = 92), resulting in an Hp1 allele frequency of 0.40. In PAOD, the Hp1 allele frequency was only 0.29, which is significantly ($P < 0.01$) different from the reference population. Relative Hp type frequencies were 0.09 (Hp 1-1; n = 11), 0.40 (Hp 2-1; n = 48), and 0.51 (Hp 2-2; n = 62). The low Hp1 allele frequency was mainly due to a strong overrepresentation of the Hp 2-2 phenotype ($P < 0.001$). In female patients, Hp1 allele frequency was 0.23; with relative Hp phenotype frequencies of 0.06 (Hp 1-1; n = 2), 0.35 (Hp 2-1; n = 12), and 0.59 (Hp 2-2; n = 20). In males, Hp1 allele frequency was 0.31; with relative Hp phenotype frequencies 0.10 (Hp 1-1; n = 9), 0.41 (Hp 2-1; n = 35), and 0.48 (Hp 2-2; n = 43). Systolic and diastolic blood pressure were comparable for the three Hp phenotypes.

In conclusion, Hp 2-2 type was overrepresented among PAOD patients. This observation is in agreement with previous clinical studies in which Hp 2-2 type has been associated with atherosclerosis. Our observation may point to a causative role of the haptoglobin polymorphism in the process of atherosclerosis.

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Regulation of lipoprotein metabolism by thiazolidine-diones occurs through a distinct, but complementary mechanism relative to fibrates

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Thiazolidinediones are antidiabetic agents, which not only improve glucose metabolism, but also reduce blood triglyceride concentrations. These compounds are synthetic ligands for PPARγ, a transcription factor belonging to the nuclear receptor subfamily of PPARs which are important transcriptional regulators of lipid and lipoprotein metabolism. The goal of this study was to evaluate the influence of a potent thiazolidinedione BRL49653 on serum lipoproteins and to determine whether its lipid-lowering effects are mediated by changes in the expression of key-genes implicated in lipoprotein metabolism. Treatment of normal rats for 7d with BRL49653 decreased serum triglycerides in a dose-dependent fashion, without affecting serum total and HDL cholesterol and apo A-I and apo A-II concentrations. The decrease in triglyceride concentrations after BRL49653 was mainly due to a reduction of the amount of VLDL particles of unchanged lipid and apolipoprotein composition. BRL49653 treatment did not change triglyceride production in vivo as analyzed by injection of Triton WR-1339, indicating a primary action on triglyceride catabolism. Analysis of the influence of BRL49653 on the expression of LPL and apo C-III, two key-players in triglyceride catabolism, showed a dose-dependent increase in mRNA levels and activity of LPL in epididymal adipose tissue, whereas liver apo C-III mRNA levels remained constant. Furthermore, addition of BRL49653 to primary cultures of differentiated adipocytes increased LPL mRNA levels, indicating a direct action of the drug on the adipocyte. Simultaneous administration of BRL49653 and fenofibrate, a hypolipidemic drug which acts primarily on liver through activation of PPARα both decreased liver apo CIII and increased adipose tissue LPL mRNA levels, resulting in a more pronounced lowering of serum triglycerides than each drug alone. In conclusion, both fibrates and thiazolidinediones exert a hypotriglyceridemic effect. While fibrates act primarily on the liver by decreasing apo C-III production, BRL49653 acts primarily on adipose tissue by increasing lipolysis through the induction of LPL expression. Drugs combining both PPARα and γ activation potential should therefore display a more efficient.

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Lack of pharmacokinetic interaction between cerivastatin, a new HMG-CoA reductase inhibitor, and digoxin

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Twenty healthy male volunteers were enrolled in a multiple-dose study to investigate the possible pharmacokinetic interaction of cerivastatin (CER), a synthetic, potent and highly selective HMGCoA reductase inhibitor, with digoxin (DIG). Subjects received a single dose of 0.2mg CER, followed 2 days later by a loading dose (Day 1) of 0.5mg DIG for 3 days, and then 0.25mg DIG daily for an additional 33 days. Concomitant dosing with 0.2mg CER daily in the evening began on Day 9 of DIG and continued for 14 days in total. DIG was administered once daily in the morning. As shown below, DIG had no significant effect on the pharmacokinetics of CER.

CER	AUC (μg.h/L)	C_{max} (μg/L)	t_{max} (h)	Half-life (h)
Day -2*	14.19	2.16	3.19	3.07
Day 9**	14.67	2.58	2.60	3.29

* single dose CER ** CER with DIG

Mean plasma DIG levels were 0.59 ng/mL on Days 6-8 (DIG-only dosing) and 0.61ng/mL on Days 20–22 (concomitant dosing of DIG and CER). Corresponding values for urine DIG levels were 94.11 and 96.09 ng/mL and for DIG clearance were 115.90 and 109.03 mL/min, respectively. CER/DIG was generally well tolerated with no serious adverse events attributable to CER. The study results indicate that during concomitant administration of CER and DIG, CER does not affect the pharmacokinetics of DIG, and DIG does not affect the pharmacokinetics of CER.

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Elderly patients with hypercholesterolaemia: results with fluvastatin

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Coronary heart disease is a major cause of morbidity and mortality in the elderly, a rapidly growing population. We evaluated fluvastatin, a fully synthetic HMG-CoA reductase inhibitor, in Caucasian patients over the age of 60 years, in 7 hospital centres. After a 4-week cholesterol-lowering diet phase, 69 patients were randomised to fluvastatin 40mg daily (33 patients) or placebo (36 patients), given for 12 weeks. 5 patients had baseline LDL-cholesterol (LDLC) 3.85 ≥ 4.00 mmol/l, and all other patients had LDLC > 4.1 mmol/l. 202 patients did not qualify for randomisation. After receiving randomised treatment for 12 weeks, 50 patients then received fluvastatin 40 mg daily on an open basis for a further 12 weeks. Mean (SD) age was 70.8(5.2) years for fluvastatin patients and 68.3(5.6) years for placebo. Mean (SD) percentage changes in lipid levels from randomisation to end of 12 weeks were as follows (N = 63, intent-to-treat analysis):

Parameter	Fluvastatin	Placebo	P value
Total cholesterol (TC) %	−21.64(8.47)	−2.91 (7.25)	< 0.01
HDL-cholesterol (HDLC) %	+4.98(10.84)	−0.05(8.68)	0.05
LDL-Cholesterol (LDLC) %	−27.14(8.45)	−2.16(9.68)	< 0.01
VLDL-Cholesterol (VLDLC) %	−30.70(30.65)	−9.80(28.6)	< 0.01

There were no statistically significant differences between treatment groups for any other biochemical or haematological parameters. Adverse events were very similar between treatment groups (fluvastatin 81, placebo 84) and no event was serious by standard criteria. Patient-assessed tolerability after randomised treatment was 'very good' for 18 fluvastatin patients and for 26 placebo patients (p = 0.79). 7 patients withdrew from the 12-week follow up (fluvastatin 4, placebo 3). We conclude that fluvastatin lowers lipid levels effectively and safely in elderly patients, producing clinically significant falls in TC, LDLC, and especially VLDLC, while increasing HDLC moderately.

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Pharmacokinetics of cerivastatin administration with and without food in the morning and evening

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Previous studies have demonstrated that some HMG-CoA reductase inhibitors show drug-food interactions—e.g. plasma lovastatin concentrations are increased by approximately 50% when given with food. This study was conducted to investigate the pharmacokinetics of cerivastatin (CER), a synthetic, potent and highly selective HMG-CoA reductase inhibitor with and without food. A total of 31 healthy male volunteers received a single oral dose of CER (0.2 mg) at weekly intervals for 4 weeks, in an open, randomised, crossover study. CER was administered according to the following schedules: in the morning following an overnight fast; with a high-fat breakfast; with the evening meal; and before bedtime. CER was well tolerated during this study. Under equivalent conditions, plasma CER levels were slightly higher in the evening compared to the morning dosing. The AUC, t_{max} and half-life of CER were not significantly different for fed versus fasting conditions either in the morning or in the evening. There was a slight (23%) but statistically significant difference between the mean C_{max} in fed and fasted subjects in the morning, but no significant difference in the evening. The study demonstrates that when CER is dosed in the evening, it can be given with dinner or at bedtime without regard to food.

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A multiple-dose study on the safety and pharmacokinetics of cerivastatin in young and elderly male volunteers

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The safety, tolerability and pharmacokinetics of cerivastatin (CER), a novel synthetic, potent and highly selective HMG-CoA inhibitor were studied in 48 young and elderly male volunteers in a randomised, double-blind, placebo-controlled study. Eight subjects aged 18–45 years (young) and 15 subjects aged 65–85 years (elderly) received CER 0.1 mg tablets daily for 7 days. The remaining (8 young and 17 elderly) received matching placebo tablets. CER was well tolerated in both elderly and young subjects. Adverse events were mainly mild and transient. The most commonly reported events were headache, dyspepsia and diarrhoea. Each of these events occurred less frequently in the CER group than in the placebo group. Overall, in those subjects given CER, the incidence of adverse events was similar for both age groups (4 of 8 young subjects, 8 of 15 elderly subjects reported adverse events). Transient and mild elevations in CPK and transaminases were evenly distributed across the CER and placebo groups. Pharmacokinetic parameters (AUC, C_{max}, t_{max} and $t_{1/2}$) were similar between the two age groups. The mean steady-state AUC on Day 7 was 5.7 μg.h/L for the young group and 6.0 μg.h/L for the elderly group. The mean peak concentrations on Day 7 were 0.87 μg/L in the young group and 1.00 μg/L in the elderly group. The mean elimination half-life for both groups was approximately 4 hours. Lipid profiles, measured at baseline and on Day 7, demonstrated a consistent lipid-lowering effect of CER compared to placebo. These results indicate that age does not affect the pharmacokinetics of CER in male subjects. CER dosed at 0.1 mg daily for 7 days was well tolerated in both young and elderly subjects.

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Picotamide reduces cardiovascular events in claudicant patients with hypercholesterolemia. An "a priori" planned sub-group analysis of the A.D.E.P. trial

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In patients at high cardiovascular risk, concomitant hypercholesterolemia may reduce the protective effect of antiplatelet drugs such as aspirin or ticlopidine. It has been shown that hyperlipemic patients are at increased risk of stroke recurrence despite aspirin treatment (*Bornstein 1994*). Picotamide is an orally active antiplatelet agent which inhibits thromboxane (Tx) A2 synthesis and antagonizes Tx receptors. The ADEP (Atherosclerotic Disease Evolution by Picotamide) trial had evaluated the effects on cardiovascular events of picotamide, in comparison to placebo, in 2313 patients with peripheral occlusive vascular disease. In the study population as a whole, picotamide reduced cardiovascular risk by 19% (P = 0.056; Logrank test). We conducted, in an "a priori" planned hypothesis, a subgroup analysis to assess the influence of concomitant hypercholesterolemia on the protective effect of picotamide on cardiovascular events in claudicant patients. The efficacy of picotamide was therefore assessed in the ADEP subgroup of hypercholesterolemic patients in order to test if the drug was still effective in this higher risk group. Hypercholesterolemia was defined as a serum total cholesterol (TC) greater than 5.2 mmol/L, measured at baseline. In picotamide-treated group, hypercholesterolemic patients were 783, whereas 262 were the patients with a TC < 5.2 mmol/L. During the 18-month of follow-up, there were 78 (9.9%) cardiovascular events (death, myocardial infarction, stroke, TIA, unstable angina, amputation) in the 783 hypercholesterolemic picotamide-treated patients as compared to 140 (13.1%) events in the 1066 placebo-treated subjects (Relative Risk Reduction: 27%, P = 0.03, Mantel-Haenszel Chi-Square test). In picotamide-treated patients, no statistical significant differences on cardiovascular event rate were observed among normo- and hyper-cholesterolemic patients (10.6% and 9.9%, respectively). In patients with peripheral occlusive vascular disease, picotamide effectively reduced cardiovascular events, irrespective to serum total cholesterol levels.

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The influence of Voglibose and Acarbose on plasma lipids in NIDDM patients in the fasting and postprandial state.

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Both, impairment of glucose and lipid metabolism are components of the insulin resistance syndrome. The aim of this study was to investigate whether the *a*-glucosidase inhibitors voglibose and acarbose influence plasma lipids in diabetics. 170 hospitalized NIDDM patients were randomly allocated to six parallel groups and received 7 days treatment with either three times daily voglibose (0.5, 1, 2, 5 mg), acarbose (100 mg) or placebo. Blood samples were taken before and 2, 3 and 4 hours after a carbohydrate rich standard meal on day -1 and day 7 to analyse phospholipids (PL), triglycerides (TG), cholesterol (C) in the serum and in VLDL, LDL and HDL as well as apolipoproteins (apo) AI, AII, B (total, VLDL, and LDL), CIII, E, and Lp(a). The effects of the treatments were analysed by one-factorial analysis of variance of the difference (day 7 minus day -1). 157 patients (mean age 54 years; 135 male) could be analysed. Fasting lipids. Voglibose decreased fasting TG (−8 % and −16% at 1 mg and 5 mg per day), VLDL-TG (−8 % and −12%), VLDL-C and VLDL-PL; none of these changes, however, was significantly different from placebo. Acarbose had no effect on fasting TG. Voglibose (5 mg) decreased apoE (−13 %, $p < 0.05$) and apoCIII (−7%, $p = 0.170$). There were no consistent changes in LDL-C, apoB and Lp(a). Voglibose did not change fasting HDL-C, but acarbose lowered HDL-C from 0.95 to 0.90 mmol/l (−6 %, $p < 0.05$). Postprandial lipids. On day -1, total TG increased in all groups by 12% on average 2 hours after the test meal. The 2 hours postprandial TG were dose-dependently reduced by voglibose (from 2.1 to 1.6 mmol/l at 5 mg, $p = 0.02$). Consistent changes were obtained with 5 mg Voglibose for VLDL-TG (−16%, $p < 0.05$), VLDL-C (21%, $p = 0.07$), VLDL-PL (−22%, $p < 0.05$), apoE (−13%, $p < 0.01$) and apoCIII (−6 %, $p < 0.05$); there were only slight reductions in the acarbose group. We conclude that voglibose is more potent than acarbose in reducing fasting TG-rich lipoproteins and the postprandial TG response.

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Audit of elderly referrals to a lipid clinic

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Current interest in routine health checks and in the burden of cardiovascular disease in old age has increased the proportion of elderly patients referred to specialist cardiovascular risk management clinics. Are these referrals justified? We have analysed patterns of referral and subsequent clinic findings for 67 male 84 female total 151 documented referrals at age 70+, range 70–92 years.

Plasma lipid profiles at presentation (mean ± 2sd) were:
Males: cholesterol 7.0 ± 3.4, triglycerides 5.1 ± 4.6, HDL 1.3 ± 0.7 mmol/l
Females:cholesterol 7.7 ± 2.7,triglycerides 3.7 ± 3.0, HDL1.5 ± 1.0mmol/l

Reason for referral was classified as:

* Routine health checks, ie no specific justification:	12/151.
* No clinical symptoms but other risk factors present:	19/151.
* Presence of active cardiovascular disease:	119/151.
* None of above but clinical signs of lipaemia eg. xanthelasma:	1/151.

Clinic findings. Other disorders with the potential to cause secondary lipaemia eg. diabetes, hypothyroid, were known at referral for 51, and at clinic were shown for a further 29, total 80/151 patients. Overweight BMI > 25 affected 61/151 (40%) of referrals. Undetected atheroma-related disease was found in 2 further patients, total 121/151 patients.

Conclusions. Some referrals came from hospital clinics, mainly vascular surgery and cardiology. Most came from family practitioners: our findings show that they have moderate but incomplete appreciation of secondary lipaemia, and the advantages of weight control supervised in family practices in the management of dyslipidaemia. Unexpectedly however the majority of referrals were justified on clinical grounds as secondary prevention in that patients had clinical atheroma-related disease. Also, the habit of routine health checks has not significantly increased the workload of elderly referrals to this clinic.

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Anti-atherogenic effect of calcium antagonist plus statin combination: amlodipine and lovastatin

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In the frame of the Regression Growth Evaluation Statin Study (REGRESS) trial synergistic anti-atherogenic effect of lipid lowering therapy with pravastatin in combination with calcium antagonists has recently been shown. Such a combination retarded progression of stenosis and reduced the number of new lesions more effectively than statin alone. In present study, we tried to elucidate the mechanism of more pronounced effect of statin-calcium antagonist combination on atherosclerotic lesion. For this purpose smooth muscle cells cultured from subendothelial intima of human aorta were incubated with whole blood serum or low density liporotein (LDL) taken from patients cotreated with lovastatin and amlodipine. As a result of the treatment, serum added to cells cultured from atherosclerotic lesion reduced cell cholesterol. Such an anti-atherosclerotic effect of cotreatment with amlodipine-lovastatin has been revealed earlier and was more pronounced compared with the effect of the treatment with amlodipine or lovastatin alone. LDL isolated from atherogenic plasma stimulated cell cholesterol accumulation. Treatment with amlodipine alone aad amlodipine-lovastatin combination lowered this atherogenic effect of LDL. As compared with amlodipine alone, combination demonstrated considerably higher anti-atherogenic effect at the LDL level. Amlodipine-lovastatin cotreatment increased sialic acid and decreased LDL susceptibility to oxidation more effectively than amlodipine alone. In addition, the combination reduced LDL negative charge while amlodipine was impotent. These findings may serve as an explanation of more pronounced anti-atherogenic effect at the lipoprotein level of amlodipine-lovastatin cotreatment compared with amlodipine alone.

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Effect of wax-matrix sustained-release niacin treatment on cholesterol esterification in plasma

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HDL-C levels negative correlate with the development of coronary heart disease(CHD). Antiatherogenic properties of HDL are related with their involving in reverse cholesterol transport. One step of this HDL-mediated process is cholesterol esterification in plasma, which catalyzed by LCAT (LCAT-reaction). It is known niacin, and its derivates induce not only decrease of total C, Tg, LDL-C, but elevation of HDL-C level.

The aim of this study was to examine the effect of wax-matrix sustained-release niacin (Endur-acin, Endurance Products Co, USA) treatment on cholesterol esterification in Plasma of men with moderate hypercholesterinemia. The study was carried out on 44 patients with CHD and 25 CHD-free subjects (aged 35–62) with plasma total C higher than 225 mg/dl after 2-month lipid-lowering diet. The patients were treated with Endur-acin: 1.5–2.0 g/day for 4 months. There were no differences in lipid and apolipoprotein levels in two groups of patients before the drug treatment, excepting HDL-C level, which was lower in CHD patients as compared to CHD-free subjects (43 ± 1.5 vs 49 ± 2.3 mg/dl, $p < 0.05$). Endur-acin treatment of CHD patients and CHD-free subjects resulted in decrease of total C by 10% and 15%, Tg by 14% and 29%, LDL-C by 16% and 19%, apo B by 20% and 25%, respectively, while level of HDL-C and apo A1 concentration increased more greater in CHD patients (19% and 16%) than CHD-free subjects (10% and 10%). The changes in rate of LCAT-reaction, which was measured by Stokke, Norum, were found as a result of Endur-acin treatment. Fractional esterification rate increased in CHD patients (from $2.64 \pm 0.20\%$ to $4.23 \pm 0.22\%$) and in CHD-free subjects (from $2.53 \pm 0.25\%$ to $3.88 \pm 0.30\%$) Molar esterification rate rose in CHD patients (43 ± 3.4 and 65 ± 3.4 mmol/l/h) and CHD-free subjects (43 ± 5.4 and 55 ± 5.4 mmol/l/h).

Thus, Endur-acin induced elevation of HDL-C was accompanied by increase of HDL-mediated plasma cholesterol esterification. This might be important in the protection of coronary atherosclerosis.

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Ability of simvastatin to inhibit DNA synthesis in PDGF-BB-, EGF-, LPA- and ET-1-stimulated smooth muscle cells.

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Smooth muscle cell (SMC) proliferation in the arterial wall plays a central role in atherosclerosis and restenosis after angioplasty. Several growth factors, such as PDGFBB, EGF, LPA and ET-1, participate in this process through the activation of various signal transduction pathways. Binding of these mitogens to their receptors results in the activation of a variety of intracellular signalling molecules and ultimately triggers cell division. Mevalonate is the precursor of isoprenoids, which are vital for diverse cellular functions, ranging from cholesterol synthesis to growth control. The characterization of proteins such as ras and related GTPases, post-translationally modified by the covalent attachment of mevalonate-derived isoprene groups (farnesyl-PP or geranylgeranyl-PP), provides new insights into the link between mevalonate pathway, signal transduction and cell cycle progression. Considering these premises, we studied the effect of HMG-CoA reductase inhibitors on growth factor-induced DNA synthesis in rat aortic myocytes. In a first set of experiments, we investigated in SMCs the effect of simvastatin (S) on [^{3}H]thymidine incorporation induced by 10% fetal calf serum (FCS). The inhibition elicited by 40 μM S ($38.9\% \pm 3.2$ of control cells; $p < 0.01$) was partially prevented by the addition of 10 μM farnesol (F) ($76.6\% \pm 3.8$) and of 5 μM geranylgeraniol (GG) ($86.8\% \pm 21.6$). Similar results were obtained when PDGF-BB (20 ng/ml) was investigated as a mitogen in cells cultured in the absence of FCS. 40 μM S inhibited PDGF-BB-induced DNA synthesis ($32.3\% \pm 3$ of control cells; $p < 0.05$), an effect partially prevented by 10 μM F ($74.2\% \pm 0.2$) and completely by 5 μM GG ($92.5\% \pm 10.3$). EGF (0.05–0.5 nM) also stimulated up to 8 fold [^{3}H]thymidine incorporation by rat SMCs; the inhibition elicited by 40 μM S ($35.4\% \pm 5.2$ of control cells; $p < 0.05$) on EGF-induced DNA synthesis (0.5 nM) was partially prevented by 10 μM F ($67.9\% \pm 6.4$) and by 5 μM GG ($65.8\% \pm 11.8$). 40 μM S inhibited LPA-induced DNA synthesis (100 μM) ($20\% \pm 11.2$ of control cells; $p < 0.05$); this effect was in part prevented by 5 μM GG ($71.1\% \pm 7.1$; $p < 0.05$), but not by F 10 μM ($27.2\% \pm 6.8$; $p < 0.01$). 40 μM S also inhibited ET-1-induced DNA synthesis (0.1 nM) ($10\% \pm 0.2$ of control cells; $p < 0.001$). Our data underline the importance of mevalonic acid metabolite(s), probably through protein prenylation (farnesylation and/or geranylgeranylation) in the regulation of cell cycle progression. A pharmacological modulation of SMCs could be useful to control one nf the maior events involved in the formation of atherosclerotic lesions.

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Treatment of Patients with Homozygous Familial Hypercholesterolemia with Expanded Doses of Simvastatin

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Homozygous familial hypercholesterolemia (HFH) is characterized by severe hypercholesterolemia and premature CHD due to defects in both LDL receptor genes. Limited treatment options are available. In a controlled, double blind, parallel, dose escalation study, the efficacy of simvastatin in HFH at doses of 80 and 160 mg/d (administered in 3 divided doses) was evaluated. After a 4 week run-in period, 12 patients with HFH were randomized to simvastatin 80 (group 1) or 40 mg/d (group 2) in a ratio of 2:1 for 9 weeks. The dose in Group I was then increased to 160 mg/d while group 2 remained on 40 mg/d for an additional 9 weeks. Baseline (BL) and mean % change from BL in lipid/lipoproteins were as follows:

	group I (n=8)			group 2 (n=4)		
	BL	80 mg/d	160 mg/d	BL	40 mg/d	40 mg/d
	mg/dl	%	%	mg/dl	%	%
T Chol	627	−23	−29	562	−12	−13
LDL-C	570	−25	−31	519	−14	−15
TRIG	136	−18	−24	72	7	−11
HDL	32	12	6	28	11	17

All 12 patients completed the trial and there were no serious or unexpected adverse events. No patients sustained significant hepatic transaminase or creatine kinase elevations. SUMMARY: Simvastatin at expanded doses of 80 and 160 mg/d was effective in reducing LDL-C in HFH.

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Clinical and genetic diagnosis of familial hypercholesterolemia in young subjects in Spain

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The diagnosis of Familial Hypercholesterolemia (FH) is based on clinical features that are not always present in childhood. DNA testing is a useful procedure for unequivocal diagnosis. The aim of our study was to compare clinical and genetic diagnosis in young subjects (< 18 years) from 19 FH families.

We studied 50 unrelated probands and 19 FH families (166 subjects). The criteria for clinical diagnosis of FH were: 1) TC and LDL-C > 95th ajusted for age and sex with TG < 200 mg/dl.2) tendon xanthomas, premature coronary heart disease (CHD) or phenotype II a in the proband or in first degree relatives. 3) Secondary hypercholesterolemia was excluded.

In the probands genetic diagnosis was performed by an initial screening for major rearrangements using Southern blot analysis (*Bg*/II). In 4 subjects we founded 4 major rearrangements and 3 complete families could be studied. From the 46 possible families without major defects we could study 16. Haplotypes for each subject were constructed by segregation analysis of 8 RFLPs from rLDL gene. ApoB3500 mutation was ruled out. From the 16 families, molecular diagnosis was confirmed using linked markers in 11, in 4 the study was not informative and in 1 family FH was not diagnosed because of non-cosegregation between phenotype and haplotypes. In the 4 not informative families a direct genetic diagnosis was performed in 2 of them.

In these 16 kindreds we studied 38 young subjects (< 18 years). A clinical diagnosis of FH was established in 23 cases. Genetic diagnoses showed that 4 individuals were clinically missclasified.

Conclusions

1) In an outbred spanish population we have confirmed clinical diagnosis of FH in 88% of the families studied.

2) Althouth we used biochemical criteria adjusted by sex and age, 10% of the young FH family members had been incorrectly diagnosed.

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Frequency and geographical distribution of familial hypercholesterolemia causing mutations in the Netherlands.

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Inherited hypercholesterolemia is caused by mutations in the low density lipoprotein (LDL) receptor gene in case of familial hypercholesterolemia (FH) or the apolipoprotein-B100 gene in case of familial defective apolipoprotein B100, which is clinically indistinguishable from familial hypercholesterolemia. In Dutch FH patients, 20 different point mutations have been described so far. To investigate the frequency and geographical distribution of FH causing mutations in the Netherlands, a cohort of 1223 FH patients from different parts of the country was analysed by PCR screening for 14 of these mutations. We detected 355 patients (29%) carrying one of these 14 mutations. We investigated the regional distribution for these 14 mutations within the Netherlands. Interestingly, 2 different mutations proved to be founder mutations. One of these founder mutations was originated in WestFriesland, a region with a geographical isolation until the 18th century.

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DNA polymorphisms of lipoprotein lipase gene in familial combined hyperlipidaemia

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The aethiology of familial combined hyperlipidaemia (FCHL) remains obscure, both genetic and enviromental factors contributing to the phenotype. FCHL is frequently associated with premature coronary heart disease (CHD). Lipoprotein lipase (LPL) is a key enzyme in lipid metabolism, and several mutations in its whole gene have been identified which can result in altered enzyme function.

The aim of the study was to screen patients with FCHL for the frequency of two common DNA polymorphisms (HindIII, PvuII) in the LPL gene by using PCR-RFLP technique.

Subjects and methods: Forty-one FCHL probands (17 females and 24 males of 8 families, age: 14–70 years) were identified by primary combined hyperlipidaemia (mean total-cholesterol (± SD): 6.7(± 1.5) mMol/L; mean triglycerides: 4.4(± 3.0) mMol/L; mean HDL-cholesterol: 1.3(± 0.3) mMol/L), elevated apoB (> 1.2 G/L), multiple type hyperlipidaemia in the family, positive family history of CHD. Forty normolipidemic age matched probands served as control group. The amplified DNA products were digested with the restriction enzymes HindIII and PvuII. Lipoproteins (total- and HDL-cholesterol, triglycerides, apolipoprotein-B) were also determinated.

Results: The frequency of HindIII genotypes in FCHL patients were: H+/H+: 0.61, H+/H−: 0.37, H−/H−: 0.02. The presence of the rare allele H− was 0.21 (vs. control: 0.25, ns). The distribution of PvuII genotypes were: P+/P+: 0.41, P+/P−: 0.45, P−/P−: 0.14. The frequency of the rare allele P− was 0.36 (vs. control: 0.45, p < .001). The highest total cholesterol (T-C) and triglycerides (TG) were in patients with H+/H+ genotype T-C: 7.3 ± 1.35 mMol/L and TG: 4.43 ± 2.35 mMol/L and with P−/P− genotype T-C: 7.49 ± 1.56 mMol/L and TG: 3.54 ± 3.21 mMol/L.

Conclusions: Our data revealed significant difference only in the frequency of PvuII genotypes in FCHL patients, however it is no association with lipids values in our study group the distribution of PvuII may be a linkage equilibrium within until now unknown mutation.

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Influence of cholestyramine on the pharmacokinetics of cerivastatin

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Objectives: To investigate the effects of cholestyramine pre-treatment at different time points on the pharmacokinetics of cerivastatin (CER).

Methods: Twelve healthy male volunteers (aged 22–35 years) received oral CER 0.3 mg with and without 12g cholestyramine (Quantalan® 50) in a four-way crossover design: A) CER at 18:00 (at beginning of dinner) B) A plus cholestyramine at 17:00 (1 h apart) C) CER at bed time, 22:00 (dinner at 18:00) D) C plus cholestyramine at 17:00 (5 h apart).

Results: CER was well tolerated. All adverse events reported were mild and transient. Cholestyramine (a bile acid sequestering agent) decreased the mean CER AUC by about 8–16% depending on the time of administration. C_{max} was reduced by about 32%, irrespective of the time of cholestyramine pre-treatment.

Cerivastatin (geom.means/SD)	AUC [µg.h/L]	C_{max} [µg/L]	t_{max}* [h]
A	20.58/1.47	3.78/1.50	1.75
B	17.23/1.45	2.61/1.44	4.0
C	22.92/1.32	4.22/1.37	2.0
D	21.16/1.39	2.87/1.34	3.0

*median

Conclusion: Cerivastatin with and without concomitant administration of cholestyramine is well tolerated. When the dosing instructions of both drugs are carefully followed, i.e. the two drugs are administered at least I hour apart, no clinically relevant interaction is anticipated.

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Deletion polymorphism of the ACE gene predicts worse left ventricular function in subjects with angiographically proven CHD below 50 years of age.

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Background: The DD genotype of the ACE gene I/D polymorphism was reported to be a risk factor of myocardial infarction and dilated cardiomyopathy. The aim of the present study was to estimate the potential association between ACE polymorphism and left ventricular volumes and function in patients with coronary heart disease (CHD).

Material and methods: The study was performed in 40 patients with CHD confirmed by coronarography, all were younger than 50 years of age (mean age 44 ± 4 years, 36 males and 4 females). Left ventricular volumes and function were measured during cardiac catheterisation. The polymerase chain reaction and agarose gel electrophoresis was used to determine the ACE I/D genotype.

Results:

	DD (n= 16)	ID (n= 17)	II (n= 7)	TOTAL
EF (ml)**	47 ± 14	61 ± 11	66 ± 14	57 ± 15
SV (ml)*	74 ± 21	98 ± 30	104 ± 42	89 ± 31
EDV (ml)	175 ± 72	157 ± 58	146 ± 53	164 ± 63
ESV (ml)*	101 ± 71	50 ± 23	42 ± 19	73 ± 55

DD vs. ID + II ** −p < 0.001, *−p < 0.02

Conclusion: DD ACE genotype is related to significantly worse left ventricular function in younger patients with coronary heart disease.

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Analysis of ace polymorphism in niddm sib-pairs concordant for normal or increased blood pressure values and albumin excretion rate.

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Nephropathy risk in diabetes appears increased by essential hypertension. Some case-control studies have indicated an association between allele D of angiotensin-converting enzyme (ACE) polymorphism and renal complication in diabetes, while others have not. We have evaluated the possible relation of ACE genotype (identified by PCR amplification of genomic DNA extracted from peripheral leukocytes) with nephropathy and hypertension in a population of NIDDM sib-pairs from North-East of Italy and Sardinia with and without hypertension and abnormal albumin excretion rate. We used the non parametric test of Bishop et al. with χ^2 test to compare the observed allele frequencies with the expected values under no linkage. We studied 46 sibships concordant for NIDDM and microalbuminuria (MA+); 116 sibships concordant for NIDDM and normoalbuminuria (MA-); 76 sibships concordant for NIDDM and hypertension (H+) and 38 sibships concordant for NIDDM and normal blood pressure values (H−). There were no significant differences in the four groups of sib-pairs concerning either allelic frequencies (MA+ expected: DD 18, ID 46, II 28 vs observed: DD 21, ID 40, II 31; MA- expected: DD 82, ID 112, II 38 vs observed: DD 77, ID 122, II 33; H+ expected: DD 55, ID 73, II 24 vs observed: DD 56, ID 70, II 26; H− expected: DD 28, ID 36, II 12 vs observed: DD 33, ID 27, II 16) or number of affected sib pairs sharing 0, 1 or 2 alleles IBS respect to expected values under no linkage (MA+ expected: X_0 1, X_1 16 and X_2 29 vs observed: X_0 0, X_1 18 and X_2 28; MA- expected: X_0 3, X_1 40 and X_2 73 vs observed: X_0 6, X_1 32 and X_2 78; H+ expected: X_0 2, X_1 26 and X_2 48 vs observed: X_0 2, X_1 24 and X_2 50; H-expected X_0 1, X_1 13 and X_2 24 vs observed X_0 1, X_1 15 and X_2 22, all p = n.s.). Our results suggest that the presence of allele D is not associated with hypertension or micro-macroalbuminuria in NIDDM sib-pairs, and that ACE genotype does not contribute to the genetic susceptibility to nephropathy and hypertension in NIDDM.

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Pharmacokinetics, safety and tolerability of multiple-dose cerivastatin in males and females: a double-blind study

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Cerivastatin (CER) is a novel, synthetic, potent and highly selective HMG-CoA reductase inhibitor effective in the treatment of hypercholesterolaemia. The safety, tolerability and pharmacokinetics of CER were studied in a randomised, double-blind, placebo-controlled trial in 49 healthy volunteers. Subjects (aged 27–81 years) were randomised into treatment groups as age-matched, male-female pairs. A total of 32 (16 female, 16 male) patients received 0.2 mg CER daily (as tablets) for 7 days and 17 received placebo. The most commonly adverse events were mild and generally equally distributed between the CER and placebo groups in both gender groups. The mean $AUC_{0\text{-}24}$ on Day 7 was 15.5 and 17.3 μg.h/L for the males and females, respectively. The corresponding mean steadystate C_{max} on Day 7 was 2.3 and 2.5μg.h/L. Half-life (3.7; 3.4h) and t_{max} (2.4; 2.7h) were similar for males and females. Lipid profiles, measured at baseline and on Day 7, demonstrated a consistent fall in total serum cholesterol and LDL-cholesterol compared with placebo in both males and females. The pharmacokinetics of CER were predictable and similar in males and females.

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Pharmacokinetics of single dose cerivastatin in subjects with normal and impaired renal function

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Cerivastatin (CER) is a novel, synthetic, potent and highly selective 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitor that effectively reduces serum cholesterol levels at low doses. Patients with renal insufficiency frequently require treatment with lipid-lowering drugs. In humans CER is exclusively cleared via biotransformation; 30% of the administered dose is excreted as metabolites in urine and 70% in faeces. To determine whether the pharmacokinetics of CER are altered in patients with renal impairment, the concentration/time profile of CER and its metabolites were studied in 6 healthy young male volunteers (Cl_{cr} > 90 mL/min/1.73 m²) and 18 subjects with varying degrees of renal insufficiency (Cl_{cr} ranged from 9 to 84 mL/min/1.73 m²) following a single oral dose of 0.3mg. No clinically significant adverse events were observed. The plasma pharmacokinetics (AUC, C_{max}, t_{max} and $t_{1/2}$) of CER and its metabolites were not altered to a clinically significant extent in patients with renal impairment, interindividual variability of the pharmacokinetic parameters was increased in the patient population. Renal elimination of the metabolites was reduced and correlated with the decrease in glomerular filtration rate, but there was no major impact on AUC, C_{max} or rate of elimination of parent compound or metabolites, suggesting that biliary excretion is unaffected. These results demonstrate that dose adjustment of CER is not required in patients with renal impairment.

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Metabolic characterization of a new form of autosomal recessive hypercholesterolemia: the FH-like hypercholesterolemia

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We have previously reported a Sardinian pedigree presenting several cases of severe hypercholesterolemia where the involvement of LDL receptor and apoB genes were excluded by haplotype segregation analysis and measurement of LDL receptor activity in skin fibroblasts in the two probands (GFC and JC) (Zuliani et al Eur. J.Clin. Invest, 1995). Several characteristics of the kindred (consanguinity, absence of vertical transmission, bimodal distribution of plasma cholesterol levels, reduction of reproductive fitness) were consistent with an autosomal recessive trait. Subsequently, we have identified a second kindred, also from Sardinia, with identical characteristics. The two probands (AP and GP) showed elevated plasma cholesterol levels (ranging between 500 and 660 mg/dl), while parents and grandparents were normal. In vitro studies ruled out defects in LDL receptor activity and binding ability of LDL. Also, beta-sitosterolemia and cholesterol ester storage disease were excluded as causes of hypercholesterolemia in this kindred. To determine the metabolic basis of this unusual inherited lipid disorder, we investigated the *in vivo* metabolism of LDL in three probands (GFC,JC,AP). First, an LDL turnover study was performed by injecting autologous ^{125}I-LDL as previuosly described (Arca et al. JAMA, 1994); five nomlocholesterolemic subjects were studied as controls. The turnover study demonstrated in all probands a severe reduction of LDLapoB catabolic rates (GFC:0. 18 poolslday. JC:0.20 pools/dav. and AP:0.22 pools/day VS 0.33 + 0.01 pools/day in normal controls) without significant alterations of production rates. Afterwards, we studied the in vivo organ-specific uptake of LDL in the three probands. 5 controls and one characterized FH homozygote. ^{99}Tc-labeled LDL were intravenously injected and the uptake of LDL by different organs was measured by γ-camera in the following 24 hours. As compared to normals, all probands showed a marked, selective reduction of LDL uptake by the liver comparable with that observed in the FH homozygous patient. Our results suggest that this unusual form of hypercholesterolemia is due to a marked reduction of in vrvo catabolism of LDL: this, in turn, is caused by a selective reduction of LDL uptake by the liver. We advance that in this new lipid disorder a recessive defect selectively causes an impairment of LDL receptor function in the liver.

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Lipoprotein lipase increases interaction of both heparan sulfate-covered surfaces and cells with oxidized lipoproteins

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The cells involved in the development of atherosclerosis lesions (endothelial cells, macrophages and smooth muscle cells) bind lipoprotein lipase (LPL) on the cell surface. This enzyme can in turn mediate cellular binding and uptake of lipoproteins. To investigate the possible participation of LPL in binding also of atherogenic lipoproteins, we have studied the effect of oxidation of human VLDL and LDL on their interaction with LPL using the surface plasmon resonance technique (BIAcore, Pharmacia Biotech, Uppsala, Sweden). LPL was non-covalently bound to heparan sulfate-covered sensor chips. Lipoproteins (non-oxidized or oxidized for different time with 20 nmoles of aqueous $CuSO_4$/mg of protein at 37°C) were passed over the sensor chip surface and their binding was continuously measured as changes in the refractive index. Moderate oxidation of lipoproteins (increase of their negative charge and accumulation of oxidation products with the absence of visible aggregation) was accompanied by a remarkable increase in binding of lipoproteins to the LPL-covered sensor chip. More extended oxidation of the lipoproteins led to aggregation, fragmentation of apolipoprotein B and to loss of the ability to interact with LPL. In experiments on THP-1 monocyte-derived macrophages similar data were obtained. Addition of LPL (10 μg/ml of medium) to the incubation medium caused an 8–10-fold increase in the binding of LDL (5 μg/ml of medium) to the cell surface. For moderately oxidized LDL this binding was 40% higher than for non-oxidized LDL. We conclude that LPL can participate in the increased binding of oxidized lipoproteins to cell surfaces. This finding may have implications for the binding of oxidized lipoproteins to cell surfaces. This finding may have implications for the binding of oxidized lipoproteins to cells *in vivo*.

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The apo CIII gene locus in familial combined hyperlipidemia. The role of the insulin response element.

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The apo AI-CIII-AIV gene cluster, located on chromosome 11, contributes to the phenotype of familial combined hyperlipidemia (FCH), but this contribution is genetically complex. Combinations of haplotypes, based on three restriction enzyme polymorphisms: XmnI and MspI sites 5' of the apo AI gene and SstI site in the 3' untranslated region of exon 4 of the apo CIII gene, were analyzed to characterize their effect on the expression of severe hyperlipidemia. An epistatic interaction was demonstrated: the S2 allele on one haplotype was synergistic in its hyperlipidemic effect to the X2M2 allele on the other haplotype. Subsequently, variants at two polymorphic sites in the insulin response element (IRE) of the apo CIII promoter (T-455C: FokI, C-482T: MspI) were examined. We show that the IRE variations itself did not affect plasma lipid variations in a population of 18 FCH families (n = 600). However analysis of combinations of haplotypes, based on these 5 polymorphisms (XmnI-MspI-SstI-FokI-MspI), revealed that the S2 allele is in strong linkage disequilibrium with the rare IRE variants and they always reside in the cis configuration. The M2 allele however, occurred both in cis and trans configuration with the IRE variant. Associations with specific combinations of haplotypes and plasma lipid variants occurred both in spouses and hyperlipidemic relatives. In the hyperlipidemic relatives (n = 10) only the 1-1-2-2-2/2-2-1-1-1 combination of haplotypes was associated with significantly elevated plasma lipid traits. In contrast in spouses signficant associations were found with specific combinations of haplotypes 1-1-1-1-1/1-1-2-2-2,2-2-1-1-1/1-1-1-2-2 (XmnI-MspI-SstI-FokI-MspI). In conclusion different susceptibility loci exist in this gene cluster, demonstrating the paradigm of complex genetic contribution to FCH.

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Stromelysin promoter polymorphism is associated with the progression of coronary heart disease

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Background: Identifying patients at increased risk for premature coronary artery disease (CAD) and more rapid progression of CAD is important because these patients might benefit from early treatment, but thus far it has proven difficult to identify such patients. Recently, an association was reported between progression of atherosclerosis and a common promoter variant, which has been shown to affect the transcription of the human stromelysin-1 gene, an enzyme involved in connective tissue turnover.
Aim: To assess whether this genetic variant confers any measurable risk for progression of CAD or for the occurrence of events and to evaluate the effects on this relation of the HMGCoA-reductase inhibitor pravastatin.
Methods: The stromelysin 5A6A promoter polymorphism was determined in 496 participants of the randomized (pravastatin vs placebo), double blind, angiographic regression trial REGRESS, investigating symptomatic patients with a total cholesterol between 4 and 8 mmol/l during a 2-year period.
Results: Patients in the placebo group with the 6A6A genotype had significantly more progression, as defined by change in minimum obstruction diameter (MOD)(0.15 ± 0.29 mm), then patients with the other genotypes (0.08 ± 0.20 mm for SASA and 0.08 ± 0.21 mm for SA6A)($p = 0.043$). In the patients treated with pravastatin, no difference in MOD change was observed ($p = 0.72$). In addition, in the placebo group a higher number of interventions ($p = 0.03$) and more new lesions ($p = 0.05$) were observed in patients with the SA6A and 6A6A genotype.
Conclusion: This study shows that the stromelysin promoter polymorphism is associated with progression of atherosclerosis and with the risk of clinical events in symptomatic patients. Treatment with pravastatin offsets the genotype effect.

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ApoE Allele Frequencies and ApoE Mutations in Endogenous Hypertriglyceridemia

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Introduction: Apolipoprotein E (apoE) plays a central role in lipoprotein metabolism by functioning as a ligand in receptor-mediated remnant removal. In the general population three major isoforms of apoE can be identified: apoE2, apoE3, and apoE4. This genetic polymorphism of apoE contributes to the normal variation in plasma lipoprotein levels. Although the effect of the apoE polymorphism on plasma cholesterol concentrations is generally accepted, the influence on plasma triglyceride (TG) levels is less well understood. Recently, we demonstrated that the apoE2-variant and other, rare, apoE mutations show decreased *in vitro* lipolysis by proteoglycan-bound lipoprotein lipase (LPL), emphasizing the importance of apoE in VLDL catabolism. In addition it has been hypothesized that the apoE isoform distribution may be different in a population of patients with hypertriglyceridemia (HTG) as compared to a normolipidemic control group.
Aim of the study: This study was designed to investigate the apoE allele frequency distribution and occurrence of apoE mutations in endogenous HTG.
Materials and methods: ApoE polymorphism was studied in 62 patients with endogenous HTG in comparison to 2018 randomly-selected 36-yrs-old males in the Netherlands. ApoE polymorphism was determined by isoelectric focusing of delipidated sera followed by immunoblotting. In case of an abnormal cysteamine modification, exon 3 and 4 of the APOE gene were sequenced in order to identify apoE mutations. ApoE phenotype and allele frequency distributions were compared by means of Fisher exact tests.
Results and discussion: The apoE phenotype frequencies in the HTG group were E2E3 21%, E2E4 5%, E3E3 34%, E3E4 37% and E3E4 3%, while in the control group they were 12.9%, 2.2%, 55.9%, 25.4% and 2.9, respectively. ApoE allele frequencies are shown in the following table.

	Hypertriglyceridemia (n = 62)	Control (n = 2018)	Significance
E*2 allele	12.9%	8.2%	$p < 0.05$
E*3 allele	62.9%	75.0%	$p < 0.05$
E*4 allele	24.4%	16.7%	$p < 0.05$

Both the apoE phenotype distribution as well as the apoE allele frequency distribution in the HTG group was markedly different when compared with a large Dutch control population. This difference is caused by increased apoE2 and apoE4 allele frequencies and a decreased apoE3 frequency in the HTG group. Of these 62 HTG subjects, two were found to have an apoE mutation, namely the apoE5(Pro^{84}→Arg;Cys^{112}→Arg) and apoE3(Arg^{251}→Gly;Cys^{112}→Arg) mutation. These mutations are rarely encountered in the general population. Screening of first-degree relatives indicate that both mutations tend to co-segregate with endogenous HTG. Both the different apoE allele frequency distribution as well as the occurrence of rare apoE mutations in this population suggest that apoE may contribute to the expresson of endogenous HTG.

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Protective effect of human apo A-II overexpression on atherogenesis in transgenic mice

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We previously generated transgenic mice expressing human apo A-II (hapo A-II: 36 ± 3 mg/dl) and described metabolic disturbances which could suggest an impaired reverse cholesterol transport process. By backcrossing for three generations to the C57BL/6 mouse strain, hapo A-II transgenic mice were developed in a susceptible diet-induced atherosclerosis background. Here, we describe the lipoprotein profile of the female animals and their atherogenesis susceptibility. On chow diet, total cholesterol was lower in transgenic mice than control mice (−15%, $p < 0.005$), solely due to a decrease of non HDL-cholesterol level in transgenic mice (−65%, $p < 0.002$). In contrast, an increase of triglyceride levels was observed in the transgenic animals (+32%, $p < 0.001$). The most characteristic change was the drop in mouse apo A-I and apo A-II levels in transgenic mice (−50%, $p < 0.0005$ and −60%, $p < 0;0007$, respectively) as compared to controls. On cholesterol rich diet, no variation of hapo AII levels was observed. However, there were decreases of non HDL-cholesterol and HDL-cholesterol levels in the transgenic group (−25%, $p < 0.007$ and −22%, $p < 0.02$, respectively) as compared to controls. HDL/non HDL-cholesterol ratios were 0.22 ± 0.06 and 0.22 ± 0.07 (NS) and total cholesterol/free cholesterol ratios were 5.27 ± 0.45 and 4.85 ± 1.24 (NS) in transgenic and control mice, respectively. Mouse apo AI level was still reduced in transgenic group (−32%, $p < 0.01$) whereas mouse apo A-II levels were not different between the two groups. After 20 weeks of diet, there was a net decrease in atherosclerotic lesions in transgenic mice compared with controls (42%, $p < 0.01$). These results suggest a protective effect of hapo A-II on atherogenesis in mice, by acting on both non-HDL and HDL metabolisms. The mechanism of this effect remains as yet unknown.

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Differential Expression of Alleles Associated with Polymorphism 54 of the Human Paraoxonase Gene

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Paraoxonase is a serum enzyme hypothetically implicated in the protection of LDL from oxidative stress. The enzyme is tightly bound to HDL and may be one source of the antioxidant capacity of this lipoprotein species. In recent studies from this lab we have: i) identified paraoxonase as a genetic risk factor for coronary heart disease, ii) demonstrated that a polymorphism affecting aminoacid 54 (L or M) is associated with highly significant differences in the plasma concentrations of the corresponding isomers. There are several possible explanations for this observation including the differences in transcription of two alleles (L and M) of the gene. In this study we examined the relative amounts of paraoxonase mRNAs corresponding to its different alleles. mRNAs were amplified by RT-PCR from human liver samples and the yields of PCR products derived from different alleles were compared with each other. To validate the approach RNA extracts from M and L homozygous livers were mixed in various proportions and subjected to RT-PCR. The relative concentrations of products measured as a ratio by densitometry corresponded to the ratios of the initial mixture of liver extracts. In the second phase the ratios of products from M and L alleles were measured in liver extracts from LM heterozygotes subjected to RT-PCR.

There was a constantly higher expression of the L allele (ratio L:M products of 1.5–3). The data suggest that there is differential expression of alleles defined by the 54 polymorphism which could explain differences in serum concentrations of the isomers.

Further studies are necessary to define the factors controlling the expression and stability of the alleles.

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Adenoviral-mediated gene transfer of macrophage colony stimulating factor to the vessel wall results in an intimal infiltrate.

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Macrophage colony stimulating factor (MCSF) is believed to play a key role in one of the earliest events in atherosclerosis, monocyte to macrophage differentiation in the arterial intima. The aim of this study was to examine the biological effects of vascular wall expression of MCSF. A recombinant adenovirus vector, encoding human MCSF (AdMCSF) was generated by standard techniques of homologous recombination in 293 cells. The rabbit carotid artery was transduced with AdMCSF by intraluminal administration of vector. As negative controls, carotid arteries were transduced either with an adenoviral vector encoding b galactosidase (AdbGal), apolipoprotein E (AdApoE) or diluent alone. Transgene expression was sought by reverse transcriptase polymerase chain reaction (RT-PCR) and immunofluorescence. The number of cross-sections with intimal infiltrates and the number of cell layers between the endothelium and internal elastic lamina were recorded. The cell type present in intimal infiltrates was analyzed by immunohistochemistry. MCSF expression was demonstrated in the vessel wall of AdMCSF-transduced vessels using RT-PCR and immunofluorescence. In contrast to control vessels, adenoviral-mediated MCSF expression was associated with an intimal cellular infiltrate consisting of smooth muscle cells and small numbers of macrophages. In AdMCSF-transduced vessels, intimal infiltrates were found in 18% of the cross-sections and more than half of these were more than 2 cell layers thick. In contrast, in control vessels, intimal infiltrates were rarely observed and were never more than 2 cell layers thick. These results suggest that MCSF may play a role in recruitment of monocytes and macrophages to the vessel wall and may contribute to smooth muscle cell proliferation and migration.

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Angiotensinogen M235T Polymorphism, but not ACE Insertion/Deletion Polymorphism is Associated with Coronary Artery Disease and Myocardial Infarction

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Gene polymorphisms encoding for components of the renin angiotensin system have been linked with an increased risk of cardiovascular disease. Therefore, we studied the relationship of the angiotensin M235T and the ACE insertion/deletion polymorphisms to the risk of coronary artery disease (CAD) and myocardial infarction (MI), respectively.

301 patients were recruited in a case-control study. Eglibility criteria included male sex, age ≤ 65 years, no acute disease ≤ 6 weeks and availability of a recent (≤ 3 months) coronary angiogram. CAD (≥ 50% stenosis in any major vessel) cases (n = 209) and controls (n = 92) were identified after blinded review of all angiograms. Genomic DNA was analyzed by polymerase chain reaction for the three possible genotypes MM, MT, or TT of the M235T variant of the angiotensinogen gene and the *II*, *ID* or *DD* genotypes of the ACE *I/D* gene polymorphism. Plasma levels of angiotensinogen and plasma ACE activity were measured by radioimmunoassay. Odds ratios and 95% confidence intervals were calculated by logistic regression.

The study population was in Hardy-Weinberg equilibrium for both genotype distributions. The presence of one or two T235 alleles was associated with a stepwise increase in mean plasma angiotensinogen concentration (no T235 allele, 14.8 ± 3.9; one, 15.7 ± 5.1; two, 17.3 ± 4.7 nmol/l; $p = 0.02$). Diastolic blood pressure in T235 homozygotes (81 ± 8 mm Hg) was significantly higher than in persons carrying only one (78 ± 8 mm Hg) or no (80 ± 8 mm Hg) T235 allele ($p = 0.03$). Assuming a recessive effect (TT vs. MT + MM), TT homozygotes had a significant increased risk for CAD (1.5 [1.1;2.1]; $p = 0.03$) and MI (1.6 [1.0;2.1]; $p = 0.05$). In contrast, although plasma ACE activity significantly depended on ACE *I/D* genotypes ($p < 0.001$), no significant increase in the risk of CAD (0.83 [0.6;1.18]; $p > 0.2$) or MI (0.9 [0.6;1.3]; $p > 0.2$) was seen in *DD* homozygotes.

In conclusion, the T235 variant of the angiotensinogen gene was associated with an increased risk of coronary atherosclerosis and thrombosis, while the *I/D* polymorphism in the ACE gene was not. These findings may indicate that a genetic variation influencing plasma angiotensinogen rather than plasma ACE activity plays a role in predisposing individuals for CAD and MI.

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Age dependent development of atherosclerosis and plasma lipids in ApoE and LDLR double knock-out mice.

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Atherosclerosis conditioned by genetic disorders in animal models progresses with age. Therefore the age of animals at start and termination of dietary intervention may be of crucial importance for the sensitivity of the model. Too short or too long duration of a study may give rise to misleading interpretation of the results. The purpose of this experiment was to obtain data on development of atherosclerosis and plasma lipids in ApoE(-/-), LDLR(-/-) mice at different age in order to determine the most appropriate duration of future dietary intervention studies. Fifty male ApoE(-/-), LDLR(-/-) mice kept on a standard diet were divided in 3 age groups and blood samples and aortic tissues were collected. Aortic atherosclerosis expressed as lipid accumulation of the aortic intima of the initial part of the ascendent thoracic aorta was quantified microscopically. The results were as follows:

Age (weeks)	Aorta, Ratio intima/wall	Cholesterol (mmol/l)					Total triglyceride (mmol/l)
		Total	HDL	LDL	IDL	VLDL	
6[a]	0.01 ± 0.01	10 23 ± 2 60	0.62	1.60	2.17	5.66	1.25 ± 0.43
12[b]	0.06 ± 0.02	11.92 ± 2.27	0.60	1.67	1.79	6.06	1.62 ± 0.53
18[b]	0.46 ± 0.17	16.58 ± 2.86	0.88	2.25	2.98	6.47	1.80 ± 0.53

Number of animals, a: 16, b: 17.

In conclusion, the results from the microscopically quantitation of aortic lipid accumulation indicate, that the degree of spontaneous atherosclerosis in ApoE(-/-), LDLR(-/-) mice increases dramatically beyond 12 weeks of age. A similar trend, although not that pronounced, were determined of total serum cholesterol.

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Chemical mapping of developing atherosclerotic plaques in APOE*3 Leiden transgenic mice by Raman spectroscopy

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Background: The chemical composition of the arterial wall is known to change during plaque development and is believed to determine whether a plaque will progress, regress or rupture. APOE*3 Leiden transgenic mice, which overexpress the dysfunctional human apolipoprotein E variant, develop hyperlipidemia and diet-induced atherosclerotic plaques, similar to those in humans. We used near-infrared Raman spectroscopy to map the chemical composition of atherosclerotic plaques at various disease stages.
Materials and Methods: APOE*3 Leiden transgenic mice (n = 35) were fed either a high saturated-fat / high cholesterol (HFC) diet or normal chow for 1, 2, 3 or 6 months. The mice were sacrificed, and their aortas (~4 mm in circumference) were flushed and cut open for spectroscopic examination. Raman spectra were obtained from locations across the luminal surface to create a grid of measurements at 0.5 mm spacing. The spectra at each location were processed to calculate the weights of cholesterol and calcium salts relative to the dehydrated weight of the artery in a spectroscopically examined volume.
Results: On the aortas of mice fed normal chow, cholesterol and calcium salts each accounted for < 3% of the relative weight at all examined locations. Plaques developed rapidly in the aorta of the mice fed the HFC diet. Mice that received the HFC diet for 6 months developed cholesterol-rich plaques with calcified cores, all located just distal to the aortic valve at the inner curvature of the arch. In this group cholesterol amounts reached maximum values ranging from 53–61%, among the different mice in the group. Calcium salts were found in the center of these plaques, at maximum values of 48–73%.
Conclusion: Raman spectroscopy can map the chemical composition of atherosclerotic plaques from transgenic mice, which could aid the study of atherogenesis. This technique may help assess hypolipidemic and antiatherosclerotic therapy.

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The effect of the obesity mutation "fatty" on hepatic apolipoprotein gene expression in rats is modified by the genetic background

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Hyperlipoproteinemia is a frequent metabolic consequence of obesity and contributes to the increased cardiovascular risk of obese individuals. Obesity mutations in rodents provide models for understanding the genetic component of obesity and its metabolic consequences. In rats the mutation "fatty" (fa) in the leptin receptor gene causes severe obesity associated with hypertriglyceridemia due to hepatic overproduction of VLDL particles with abnormal apolipoprotein composition. In an earlier study we showed that the fa mutation expressed on the background of the Zucker rat strain (fa/fa Zucker fatty rats) leads to hepatic overexpression of apoA-IV due to a nuclear posttranscriptionai mechanism, whereas apoA-I expression and apoC-III mRNA were unaltered (1). Moreover, transcriptional downregulation of the apoA-IV gene by a fish oil diet was abolished specifically in (fa/fa) Zucker fatty rats. Transcriptional downregulation of the apo A-I gene occurred both in lean (Fa/-) and fatty (fa/fa) animals.
To determine, whether these effects of the fa mutation on hepatic apolipoprotein gene regulation are modified by the genetic background of the rat strain, we now studied the effect of fa on hepatic apoA-IV, A-I, and C-III mRNA and nuclear RNA abundance in Wistar rats by Nothern and quantitative slot blotting. ApoA-IV and apoA-I transcription rates were determined by run-on assays.
In (fa/fa) Wistar fatty rats total cellular apoA-IV mRNA was increased to 398 ± 17 %, nuclear apoA-IV RNA was increased to 330 ± 47% of the values in lean (Fa/-) controls (100 ± 18 and 100 ± 46 % respectively, $p < 0.05$, 6 rats/group), whereas apoA-IV gene transcription was not enhanced. This suggests posttranscriptional upregulation of the apoA-IV gene in the liver of Wistar fatty rats, as observed earlier in Zucker fatty rats. In contrast to Zucker fatty rats, basal apoA-I gene transcription, apoA-I nuclear RNA and total cellular RNA were reduced to half of lean control values in Wistar fatty rats. Hepatic apoC-III mRNA was increased to 174 ± 15% (6 rats/group, $p < 0.05$) in Wistar fatty rats vs. lean controls, whereas no effect of fa on hepatic apoC-III mRNA was found in the Zucker strain. Unlike in Zucker fatty rats, a fishoil diet (20% Menhaden oil for 3 weeks) reduced hepatic apoA-IV transcription from 117 ± 27 to 46 ± 15 % and apoA-IV nuclear RNA to 64 ± 14% of lean control values levels in Wistar fatty rats. This response was blunted by posttranscriptional mechanisms and total cellular apoA-IV mRNA remained unchanged. In contrast our earlier results in Zucker fatty rats, apoA-I gene transcription, nuclear RNA and mRNA in Wistar fatty rats were not reduced by fish oil diet. No effect of dietary fish oil on hepatic apoC-III mRNA abundance was found in either rat strain .
Our data indicate that the effects of a given obesity mutation on basal and diet induced hepatic apolipoprotein gene expression can be modified considerably by factors from the genetic background on which the obesity mutation is expressed.
1) Strobl, W. et al. J. Clin. Invest. 92:1766–1773 (1993)

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Use of scavenger receptor regulatory elements to target gene expression to atherosclerotic plaques.

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The development of macrophage foam cells within atherosclerotic lesions is thought to depend on the expression of one or more scavenger receptors that mediate the uptake of oxidized lipoproteins. We have cloned and characterized genomic regulatory elements that control the expression of the scavenger receptor A gene. These elements confer macrophage-specific expression of linked genes in vivo, and are capable of directing high levels of transgene expression in foam cells of atherosclerotic lesions. Intriguingly, sequence elements that are sufficient to direct expression in peritoneal macrophages and mediate M-CSF dependent expression in bone marrow-derived macrophages are not sufficient to direct expression in atherosclerotic lesions. High levels of expression in lesions appear to require the presence of additional regulatory elements that mediate transcriptional responses to GM-CSF, and which functionally interact with M-CSF response elements. We and other laboratories are currently utilizing SR-A regulatory elements to investigate the consequences of overexpression of various genes on the development of atherosclerosis. In addition, we have recently cloned and characterized genomic regulatory elements for macrosialin, the murine homologue of cd68. The macrosialin promoter is approximately 200 times stronger than the SR-A promoter in macrophage cell lines, suggesting that it may offer some advantages over the SR-A promoter in experiments requiring high levels of transgene expression.

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Lipoprotein metabolism and atherosclerosis in APOE*3Leiden mice.

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APOE*3Leiden transgenic mice develop hypercholesterolemia after feeding cholesterol containing diets. In contrast, transgenic mice carrying the APOE2(arg158→cys) variant (apoE2) do not display hyperlipidemia. This confirms the common assumption that apoE2 is a recessive variant, whereas with apoE3Leiden the hyperlipidemia is dominantly inherited. Introducing these two apoE variants onto an endogenous apoE deficient background showed that apoE3Leiden is able to rescue the extreme hyperlipidemia in apoE-/- mice, whereas apoE2 is not. In vitro analyses showed that apoE3Leiden containing VLDL still displays LDLR binding activity, whereas apoE2 VLDL is completely defective in this respect. In both apoE2 x apoE-/- and apoE3Leiden x apoE-/- mice serum lipid levels strongly increased after Ad-RAP transfection, indicating that both apoE2 and apoE3Leiden bind to the LRP. In addition, Ad-VLDLR transfection in the liver of these two mouse lines leads to a decrease in plasma lipid levels, indicating that these apoE variants also bind to the VLDLR.

APOE*3Leiden transgenic mice are highly susceptible to diet-induced atherosclerosis. Using quantitative image analysis we investigated the effect of diet composition and feeding period on serum cholesterol and atherosclerotis in the aortic sinus in these mice. The atherosclerotic lesion areas in cross sections were 5 to 10 times greater in transgenic mice compared with non-transgenic littermates. Highly significant positive correlations were found between the log-transformed data on lesion area and serum cholesterol exposure ($r = 0.85$), indicating that hypercholesterolemia is likely to be a major determinant in lesion formation. We conclude that the APOE*3Leiden mouse represents a promising model for research on hyperlipidemia and atherosclerosis development.

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Lack of aneurysm formation in atherosclerotic aorta from plasminogen activator deficient mice due to reduced metalloproteinase activity.

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Impaired plasmin proteolysis has been implicated in progression of atherosclerosis by promoting fibrin and matrix deposition, but recent evidence has suggested a role for increased plasmin proteolysis in plaque ulceration, rupture and neovascularization, and in aneurysm formation, possibly via interaction with metalloproteinases (MMP). In order to investigate the role of the plasminogen activators (PA), tissue-type PA (t-PA) and urokinase-type PA (u-PA) in atherosclerosis, mice with a deficiency of t-PA (tPA-/-) or u-PA (u-PA-/-) were intercrossed with apolipoprotein E deficient (apoE-/-) mice and fed a cholesterol rich diet for prolonged periods. There were no genotypic differences in the plaque area nor in their anatomical location across the aorta. However, the media in plaques from apoE$^{-/-}$ or apoE$^{-/-}$:t-PA$^{-/-}$ mice displayed different stages of destruction, ranging from fragmentation of the elastic laminae to transmedial perforation and microaneurysm formation. Ultrastructural analysis revealed degradation of elastin and collagen. In contrast, the media in apoE$^{-/-}$:u-PA$^{-/-}$ arteries were protected against such destruction, and displayed only a minor degree of fragmentation of the elastic laminae. Plaque macrophages expressed abundant u-PA mRNA, immunreactivity and zymographic activity. When cultured in the presence of plasminogen, wild type, t-PA$^{-/-}$, MMP-3$^{-/-}$, MMP-7$^{-/-}$ and MMP-9$^{-/-}$ but not u-PA$^{-/-}$ or MMP12$^{-/-}$ macrophages degraded 3H-elastin. Activation of MMP-3, MM-9 and MMP12 was increased by culturing wild type and t-PA$^{-/-}$ but not u-PA$^{-/-}$ macrophages in the presence of plasminogen. Immunostaining revealed the presence of MMP-3, MMP-9 and MMP-12 in plaque macrophages. Taken together, these results suggest that u-PA, via generation of plasmin, activates elastolytic as well as collagenolytic proteinases, mediating thereby destruction of the media and aneurysm formation.

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A role for interferon-γ but not interleukin-4 in graft arteriosclerosis

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Differential contributions of T_H1 (e.g., interferon-γ [TFN-γ]) versus T_H2 (e.g., interleukin-4 [IL-4] cytokines likely modulate the development of acute parenchymal rejectoin and graft arteriosclerosis in solid tissue transplants. To evaluate the relative importance of these cytokines, we have used genetically-deficient mouse strains lacking IFN-γ or IL-4 as recipients in a vascularized heterotopic murine cardiac allograft model. Total MHC-mismatched- or MHC class II-disparate hearts were transplanted into wild-type, IFN-γ deficient (GKO), or IL-4-deficient recipients with or without anti-CD4 and anti-CD8 monoclonal antibodies for immunosuppression.

In the absence of prior immunosuppression, total MHC-mismatched grafts in wild-type or GKO hosts all showed similar rates of graft failure, manifested histologically as severe acute rejection. However, grafts into GKO recipients characteristically ceased beating 1-2 days earlier than wild-type grafts; in addition to focal acute rejection, the grafts in GKO recipients exhibited profound coagulation necrosis with multiple arterial thrombi, suggesting a role for IFN-γ in locally modulating thrombosis.

In total MHC-mismatched grafts, long-term graft survival (at least 12 weeks) could be achieved by weekly anti-CD4 and anti-CD8 administration after an initial 4 day course of rejection. Similar long-term graft survival was obtained in MHC II-disparate combinations when the hose received anti-CD4 and anti-CD8 before transplant only. In such long-term transplants of either strain combination, grafts into wild-type hosts reliably developed vascular lesions of graft arteriosclerosis by 8–12 weeks. In comparison, grafts into GKO recipients developed no graft arteriosclerosis, despite a severe ongoing parenchymal infiltrate comparable to wild-type grafts. A similar lack of graft arteriosclerosis, even in the presence of parenchymal rejection, was seen in MHC II-disparate transplants into wild-type recipients given weekly injections of anti-IFN-γ antibodies. Although grafts in GKO recipients showed numbers of macrophages, CD4, and CD8 cells comparable to grafts in wild-type recipients, grafts into GKO recipients had diminished MHC II, CD40, VCAM-1 and ICIAM-1 expression relative to wild-type controls. In contrast, MHC II-disparate transplants in IL-4 deficient animals showed parenchymal rejection and graft arteriosclerosis at rates and severities comparable to wild-type controls.

Thus, deficiency of IL-4 does not affect the progress of acute rejection or graft arteriosclerosis in cardiac allografts. However, IFN-γ is important to the development of graft arteriosclerosis, despite the fact that lack of IFN-γ does not diminish acute parenchymal rejection and graft failure, IFN-γ may mediate graft arteriosclerosis by mechanisms involving secondary activation of macrophages, or expression of MHC, adhesion, or accessory molecules.

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Overexpression of human apolipoprotein A-I in transgenic rabbits

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Cholesterol in High density lipoproteins (HDL) and apolipoprotein (apo) A-I levels are inversely correlated with coronary heart diseases. Overexpression of human apoA-I in several strains of mice has been shown to protect against atherosclerosis development. However, lipoprotein metabolism in mice are far from that in human. Concerning this aspect, the rabbit model presents several advantages with the presence of cholesteryl ester transfer protein or with the apoB-containing lipoproteins as major cholesterol carriers in plasma. Therefore, in order to study the role of apoA-I in lipoprotein metabolism and assess its efficacy to retard atherosclerosis progression in another animal model, we produced human apoA-I transgenic rabbits (New Zealand White background, NZW) with the use of an 11-kb genomic apoA-I fragment containing a liver-specific promoter. Transgenic animals expressed human transgene in the liver whereas, in rabbits, apoA-I is normally produced by the intestine. In one of five transgenic lines established, human apoA-I concentration was superior to 175 mg/dl whereas rabbit apoA-I levels were significantly reduced. In this line, HDL-cholesterol levels were 2-fold increased in comparison with that of non transgenic littermate. Protective effect of apoA-I overexpression was evaluated in rabbits fed a cholesterol rich diet for 14 weeks. Plasma levels of atherogenic apoB-containing lipoproteins (>1000 mg/dl) were matched between transgenic and control animals. HDL-cholesterol levels in transgenic rabbits were still approximately twice that of the control rabbits. At the end of the experiment, the amount of aortic surface area covered by the lesions as well as the amount of lipid accumulation in the aorta were significantly less in transgenic rabbits compared with control rabbits, confirming the beneficial properties of apoA-I-containing lipoproteins. Furthermore, human apoA-I transgenic rabbits were crossed with Watanabe rabbits, a relevant model of familial hypercholesterolemia, which develop spontaneously severe atherosclerosis. Human apoA-I levels in human apoA-I Watanabe rabbits, 101 mg/dl, were lower than that in transgenic NZW rabbits. Rabbit apoA-I levels were similar between human apoA-I Watanabe rabbits and control rabbits. However, HDL-cholesterol concentration were still 2-fold higher in transgenic versus non transgenic Watanabe rabbits. These data established the fundamental role of apoA-I in HDL metabolism and in the atherosclerosis process.

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Use of adenoviral vectors to increase reverse cholesterol transport.

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Adenovirus-mediated gene transfer by systemic administration of recombinant replication-defective adenoviruses results in a highly efficient transduction of hepatocytes in vivo. Major advantages of gene transfer by adenoviruses are the capability to transduce both replicating and non-replicating cells and the easy production of adenovirus stocks of high titer. The most important limitation of current technology is the transient nature of transgene expression which is principally due to an immune response against the transgene and viral gene products.

Potent transient effects on lipoprotein metabolism have been demonstrated using recombinant adenoviruses expressing the LDL receptor, the VLDL receptor, 7 α-hydroxylase, apo E, APOBEC-1 and a transdominant negative mutant of APOBEC1. A recombinant adenoviral vector containing the rabbit LDL receptor cDNA not only lowered LDL cholesterol levels, but also increased HDL cholesterol levels and apo A-I levels by 300–400% in Watanabe Heritable Hyperlipidemic rabbits. Administration of an adenovirus containing the human apo E gene to apo E deficient mice led to a normalisation of total cholesterol levels and a more than threefold increase of HDL-cholesterol. Using an adenoviral vector expressing human apo A-I under control of the CMV-promoter, we found a 2.8-fold increase of HDL-cholesterol levels in C57BL/6J mice and a 2.7-fold increase of HDL cholesterol in C57BL/6J apo E deficient mice after administration of 4 x 10^9 p.f.u. The HDL cholesterol / non-HDL cholesterol ratio increased from 0.063 ± 0.003 in control apo E deficient mice to 0.16 ± 0.015 three days after injection. Human apo A-I levels were nearly undetectable 3 weeks after injection. Previously, introduction of a human apo A-I transgene in apo E deficient mice has been shown to be a potent suppressor of atherosclerosis. Similarly, the transient increase in HDL cholesterol levels induced by adenovirus mediated gene transfer of human apo A-I in apo E deficient mice led to a profound reduction in neointima formation after endothelial denudation of the common carotid artery.

Because of the potent anti-atherogenic effects of human apolipoprotein A-I as shown in transgenic mice, the clear inverse relationship between HDL cholesterol and cardiovascular disease in humans and the absence of a relationship between HDL cholesterol and non-cardiovascular disease, adenovirus mediated gene transfer with apolipoprotein A-I is particularly attractive. However, the transient nature of gene expression remains the major

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Adenoviral-mediated gene transfer of endothelial nitric oxide synthase to the rabbit carotid artery alters vascular reactivity.

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We tested the effect of overexpression of endothelial nitric oxide synthase gene in the rabbit carotid artery by adenoviral mediated gene transfer. Rabbit carotid arteries were surgically isolated and exposed to an adenoviral vector encoding eNOS (AdeNOS) and an adenoviral vector encoding ß-galactosidase on the other side (ß-Gal). Vector solutions at a concentration of 10^{10} pfu/l were instilled intraluminally for 20 minutes or delivered to the periadventitial sheath of the vessel. Arteries were harvested 4 days later for immunostaining, vasomotor studies and measurement of cGMP levels and NOS activity. Intraluminal administration resulted in endothelium specific gene transfer, while adventitial delivery resulted in adventitial specific gene transfer. In both delivery models cGMP levels were significantly higher in the AdeNOS transduced vessels. Maximal contractions to phenylephrine were diminished in both models (Intraluminal: 6.0 ± 0.3 grams vs 5.0 ± 0.2 grams, $p < 0.02$; Adventitial: 5.6 ± 0.2 grams vs. 4.6 ± 0.2 grams, $p < 0.05$.). This effect was abolished in the presence of the NOS inhibitor L-NMMA. After intraluminal administration, relaxations to acetylcholine obtained during submaximal contractions to phenylephrine were significantly enhanced in the AdeNOS arteries ($-\log EC_{50}$ 7.4 + 0.08vs 7.09 + 0.09, $P < 0.02$). After adventitial administration, relaxations to calcium ionophore obtained during submaximal contractions to phenylephrine were significantly enhanced in the AdeNOS vessels ($-\log E_{50}$ 7.77 ± 0.08 vs 7.45 ± 0.07, $P < 0.02$. We conclude that overexpression of eNOS in the endothelium or adventitia results in altered vasoreactivity. These results suggest that gene transfer of eNOS may be beneficial in the treatment of vasospasm and endothelial dysfunction.

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Metabolic role of apolipoprotein A-II studies in transgenic mice

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High density lipoprotein (HDL) levels are inversely correlated with the incidence of atherosclerotic cardiovascular diseases. HDL may protect against atherosclerosis by promoting transport of excess cholesterol from peripheral tissues to liver, a process termed reverse cholesterol transport (RCT). In addition, HDL protects low density lipoproteins from oxidative damage known to contribute to the onset and progression of atherosclerosis

The major HDL proteins are apolipoprotein (apo)A-I and apoA-II. Some evidence suggests that apoA-I is directly protective against atherosclerosis, but relatively little is known about the role of apoA-II. Contrasting results have been obtained in mice overexpressing human (h) or murine (m) apo A-II.

hapoA-II, a homodimeric protein, seems to confer protection with regard to atherogenesis, in contrast, overproduction of mapoA-II (that is monomeric) has been proposed to be atherogenic. To further investigate the role of apoA-II we measured the initials steps of RCT and the paraoxonase activity in both types of mice. To determine if the observed differences were related to the mono or dimeric forms we studied transgenic mice for hapo A-II rendered monomeric by (Cys6→Ser) mutation (hapoA-IImon).

mapoA-II mice had cHDL values 84% higher than control mice, their serum or HDL had a capacity to promote cellular cholesterol efflux that was twice the one of controls. LCAT activity measured with exogenous substrate was not different to the activity of control sera (31.8 ± 1.8 vs 33.8 ± 2 %) but the endogenous esterification capacity was higher (16 ± 2.5 vs 7.2 ± 0.2 %). hapoA-II mice with normal cHDL and hapoA-IImon with reduced cHDL had lower capacity to efflux cholesterol and to esterify it than control mice.

Paroxonase activities for mapoA-II, hapoA-II and hapoA-IImon were respectively 98, 83 and 73% of control mice serum activity

Our results are a clear indication that the metabolic differences observed between m and h apoA-II are not related to the mono or dimeric forms, and above all that RCT is a more complex pathway and that increased activity of some steps is not a proof that peripheral cholesterol will be efficiently eliminated.

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EUROASPIRE: A European Society of Cardiology survey for the potential for secondary prevention of coronary heart disease in nine European countries.

EUROASPIRE Investigators
European Society of Cardiology Working Group on Epidemiology and Prevention Joint ESC/EAS/ESH Implementation Group on Prevention of Coronary Heart disease

The potential for secondary prevention of coronary heart disease in Europe was measured in a survey of nine European centres (Czech Republic, Finland, France, Germany, Hungary, Italy, Netherlands, Slovenia, Spain) on behalf of the Working Group on Epidemiology and Prevention of the European Society of Cardiology.
Consecutive patients (age ≤ 70 years) in each of four diagnostic groups — coronary artery bypass graft, percutaneous transluminal coronary angioplasty, acute myocardial infarction and acute ischaemia without myocardial infarction — were identified from centres. Coronary risk factors were abstracted retrospectively from medical notes and patients were then invited for interview and examination at least six months after the acute procedure or event. The final number of records reviewed was 4866, and 3569 (73%) patients attended for interview.
In all centres and diagnostic categories combined, 19.4% were cigarette smokers at interview; 25.3% had a body mass index ≥ 30 kg/m2; 45.6% had blood pressure level in the limits of mild hypertension and 7.5% of severe hypertension; 44.0% had a total cholesterol ≥ 5.5 mmol/l; 18.0% were diabetic; and 52.5% had a positive family history of coronary heart disease. Reported drug therapy was as follows: 81.2% antiplatelets; 53.7% beta-blockers; 29.5% ACE inhibitors; 32.0% lipid lowering drugs.
Across Europe there is still considerable variation in the distribution of risk factors between both centres and diagnostic categories at the time of interview. Moreover, although the use of prophylactic drug therapy in coronary patients is based on scientific evidence from randomised control trials common to all European countries there is striking variation in the use of different classes of drugs between centres, except for the use of antiplatelets.
In patients with coronary heart disease in Europe there is considerable medical potential to reduce the risk of a further major ischaemic event with a comprehensive secondary prevention programme.

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Prevalence of macrovascular complications and its consequences for lipid lowering treatment in patients with diabetes mellitus type II

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Background: Coronary heart disease (CHD) and other manifestations of macrovascular disease are important causes of morbidity and mortality in patients with diabetes with rates being 2–4 times higher than in non-diabetics. Several risk factors have been identified among which hypertension, glycemic control, smoking, insulin-resistance and dyslipidemia. In several large scale trials, including the 4S study and the CARE study the beneficial effect of LDL-cholesterol lowering therapy by HMG CoA-reductase inhibitiors, has been well demonstrated. Moreover, post-hoc analysis of the 4S study showed that in the small group of patients with diabetes there was a significant decrease in cardiovascular morbidity and mortality. The impact of these results in the management of care in diabetic outpatient-clinics is however unknown. This study was performed to assess the prevalence of macrovascular complications and its consequences for lipid lowering treatment in patients with DM II in order to evaluate the impact on clinical practice of the results of the 4S study.
Methods: From February 1995 till November 1996 the medical history of 1198 patients with DM II of a large non-academic teaching hospital was stored into a computerized database with specific attention to the following: presence of macrovascular disease, dyslipidemia and the use of lipid lowering drugs.
Results: The mean age was 65 years, 65 % of the patients used insulin and the mean HbAlc was 7.7 ± 1.6 %. Hypertension was present in 39% and treated accordingly in all patients. Macrovascular complications were present in 40% of the patients, with a prevalence of symptomatic coronary artery disease (CAD) of 31%, peripheral artery disease of 9.8 % and cerebrovascular disease (CVA and/or TIA) of 11.2%. The mean plasma total cholesterol and triglycerides were 5.9 (SD 1.2) and 2.43 (SD 1.9) mmol/l respectively. Surprisingly, lipid lowering drugs were prescribed in only 6.5 % of the patients. Moreover, 9.6 % of the patients with macrovascular disease used lipid lowering treatment, while this number was 4.7% in the patients without macrovascular disease.
Conclusion: This study shows that within a setting of an outpatient clinic of a large non-academic teaching hospital, in contrast to a well established program of glycemic control and hypertension treatment, only 9.6 % of the DM II patients with clinically apparent macrovascular disease were prescribed lipid lowering drugs in a period of two years following the publication of the 4S study.
It is concluded that education programs both to DM II patients and their physicians are urgently needed to fill in this treatment gap.

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The costs of Lipid Lowering: What are the problems now?

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The efficacy of lipid lowering drug therapy in reducing rates of coronary heart disease (CHD) has been clearly established, particularly with new, more potent, lipid lowering drugs. Data has become available from major population intervention trials in both primary and secondary prevention, and also from coronary atheroma regression studies.
Efficacy applies to hyperlipidaemic subjects with and without CHD, but cost-effectiveness is influenced by other factors, such as the absolute population risk for CHD, the drug cost per unit of cholesterol lowering (and therefore of CHD rate reduction), the delay time between intervention and change in outcome, and the age or potential life expectancy of the person treated.
In secondary prevention, treatment is likely to be cost-effective in most circumstances, if there is adequate life expectancy. Is there a lower limit for LDL-cholesterol for intervention or goal?
In primary prevention CHD rates in familial hypercholesterolaemia usually warrant treatment, but what absolute risk (1, 2, 5, 10% per annum?) of CHD should be applied to the general population? Many patients need to receive treatment to achieve CHD prevention in some. Moderate benefit may be considered to be at too high a price by a purchaser other than the patients themselves.
How much should psychological aspects of treatment influence decisions with the 'medicalisation' of the asymptomatic? How do we factor in other risk factors (eg. triglycerides, HDL) into decision making?
CHD prevention by lipid lowering has arrived. How much has to be decided.

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International comparison of awareness and attitudes towards coronary risk factor reduction: the HELP Study

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A large scale international survey was conducted to assess public attitudes to coronary health and to establish the frequency with which certain health-related behaviours are practised in five European countries.
Representative individuals from the general public (n = 5013); individuals at increased risk of coronary disease (n = 2500); patients who had suffered a myocardial infarction (n = 1256) and members of their families (n = 1249) were interviewed in a study conducted in France, Germany, Italy, Sweden and the United Kingdom. Questions were asked about respondents' attitudes to their health, and about their current health practices. Their level of satisfaction with the health-related information that they received and its perceived credibility was analysed according to its source. The survey revealed a considerable degree of indifference to coronary heart disease, despite the possession of a reasonable level of knowledge of the risks involved, even among patients who had suffered a myocardial infarction. For example, only a third of the general public could define an "acceptable" level of blood cholesterol. A the same time, respondents declared themselves satisfied with the quality of advice about coronary health which they obtained from the medical profession and regarded these sources of information as highly credible. Media health campaigns, by contrast, had comparatively little impact.
Individuals therefore possess a reasonable knowledge about coronary disease. They also receive heart health information which is perceived as highly credible. Nonetheless it has a very limited impact on their actual practice of health-related behaviours. There is an urgent need to develop new methods for the effective delivery of healthcare messages.

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Abstract not received

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Economic analysis of lipid lowering cost in Belgium

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Aim. Methodological differences and variations in health care regulations among countries often preclude direct comparisons of cost-effectiveness studies. We have applied a projected risk model, designed to determine the economic value in the United States of pravastatin in the secondary prevention of coronary heart disease (CHD), to Belgium using local health care costs.
Methods. A Markov process was used to model the effectiveness of treatment for three years with pravastatin vs. placebo in 1,000 male CHD patients aged 60 years and clinically similar to those in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC I) and Pravastatin, Lipids and Atherosclerosis in the Carotid Arteries (PLAC II) studies. The PLAC I and II trials have shown that pravastatin treatment for three years at a weighted mean dose of 36.64 mg daily significantly reduced the incidence of non-fatal myocardial infarction in patients with CHD. Framingham data were used to project the risk of mortality 10 years post-myocardial infarction.
Results. The incremental cost per life year gained (LYG), alter discounting costs and benefits by 5% annually, in the setting of Belgian health care regulations was BEF 720,794 (US$ 24,359) for CHD patients with one additional risk factor; BEF 526,464 (US$ 17,792) for those with two additional risk factors, and BEF 392,765 (US$ 13,274) for those with three or more additional risk factors. The cost per LYG in Belgium appeared to be more sensitive to drug acquisition cost than to costs of medical interventions.
Conclusion. The cost-effectiveness ratios of pravastatin monotherapy for three years in secondary prevention of CHD, obtained with the same projected risk model, are from 86% to 92% higher in Belgium than in the United States, due to differences in medical patterns of practice and in intervention costs.

XIth International Symposium on Atherosclerosis

The **XIth** International **S**ymposium on **A**therosclerosis will be held on **October 5 - 9** in the Palais des Congrès of **PARIS**, under the co-chair of B. Jacotot and J.C. Fruchart. This Symposium, organized every 3 years (the first time in France), is the major international event in this field.

The Symposium will meet scientists and physicians from all around the world, on the following topics :

- **Arterial wall biology**
- **Athero-thrombosis**
- **Genetic and geographical epidemiology**
- **Prevention of atherosclerosis and its risk factors**
- **Molecular biology of lipoprotein disorders**
- **Genetics of atherosclerosis and dyslipidemias**
- **Insulin resistance, diabetes mellitus and atherosclerosis**
- **Assessment of atherosclerosis lesions, progression and regression**
- **Aging and atherosclerosis**
- **Nutrition and ischemic diseases**
- **Drugs affecting lipid metabolism and atherosclerosis**

Six satellite Symposia will be held before or after the major meeting :

- **Reverse cholesterol transport** (Lille Oct. 2-4).
  Chair : J.C. Fruchart and H. B. Brewer
- Atherogenic LDL : **Physiopathology and new therapeutic approaches** (Abbaye of Royaumont Oct. 2-4).
  Chair : M.J. Chapman and D. Steinberg
- **Haemostatic variables in atherothrombosis : Prediction and Prevention** (Marseille Oct. 2-4).
  Chair : I. Juhan-Vague, G. Lowe and L. Drouet
- **Smooth muscle cell proliferation and differentiation** (Bordeaux Oct. 2-4).
  Chair : J. Bonnet and J.M. Isner.
- **Lipoprotein metabolism, Obesity and Atherosclerosis** (Saint-Malo Oct. 3-4).
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Pathophysiology of Hypertension

Edited by **A. Zanchetti** and **G. Mancia**, *Centro di Fisiologia Clinica e Ipertensione, Università di Milano e Ospedale Maggiore, Milan, Italy*

Handbook of Hypertension Volume 17

This new edition of *Pathophysiology of Hypertension* in the series of the volumes of the *Handbook of Hypertension* has been designed in an entirely different way to cope with the developments in the field that have occurred in the decade between 1987 and 1997. The two separate volumes of the previous edition have been joined in a single, though much thicker volume to epitomize the close connections between structural and functional aspects of hypertension, a concept that has marked progress in our understanding of the pathophysiology of hypertension in the last decade.
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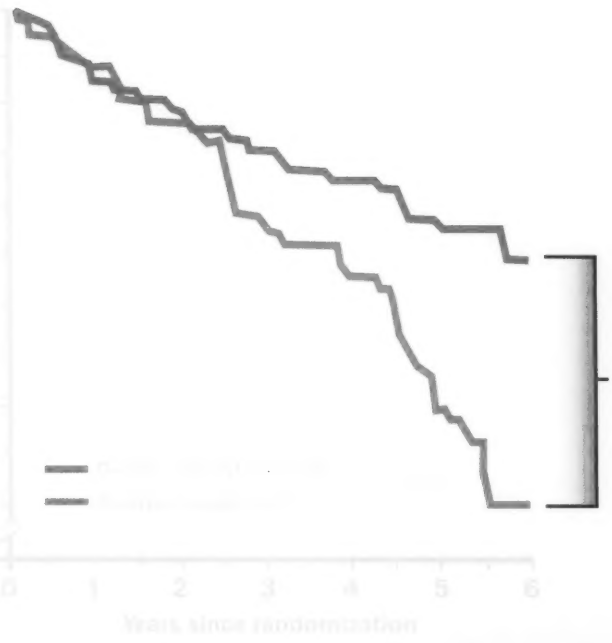

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